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VOL. 46 DECEMBER, 1955 NO. 6

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Clinical and Experimental

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The Journal of Laboratory and Clinical Medicine

VOL. 46 DECEMBER, 1955 No. 6

CLINICAL AND EXPERIMENTAL

SERUM GLUTAMIC OXALACETIC TRANSAMINASE AS AN INDEX OF HEPATOCELLULAR INTEGRITY

DAVID W. MOLANDER, M.S., M.D., FELIX WRÓBLEWSKI, M.D., AND JOHN S. LADUE, M.D., PH.D.
NEW YORK, N. Y.

GLUTAMIC oxalacetic transaminase (GO-T) is a specific enzyme concerned with the transfer of the alpha amino nitrogen of aspartic acid to alpha ketoglutaric acid, resulting in the synthesis of a new amino acid, glutamic acid, and a new alpha keto-acid, oxaloacetic acid (Fig. 1). This protein catalyst is widely distributed in animal tissues but its concentration or activity is greatest in heart muscle, skeletal muscle, brain, liver, and kidney in decreasing order.[1]

The relatively high GO-T activity in liver tissue suggested to us that liver-cell injury might result in a release of this enzyme into the serum comparable with that already reported following acute myocardial infarction.[2] None of the liver function tests presently available provides a useful index of the degree of liver-cell injury or destruction.

The following experiments suggest that serum glutamic oxalacetic transaminase activity (SGO-T) seems to be an extremely sensitive index of hepatocellular integrity. The extent of any necrotizing process upon the liver parenchyma appears to be reflected in the level of enzyme activity. The day-to-day change of SGO-T apparently parallels the degree and extent of injury as well as the rate of progress in attaining restoration of normal hepatocellular function following acute injury to the liver cells.

From the Sloan-Kettering Division of Cornell University Medical College and Memorial Center, New York, N. Y.
Received for publication June 27, 1955.

The striking elevation of SGO-T activity in two patients exposed to CCl_4 two days before hospital admission is shown in Figs. 2 and 3. The enzymatic activity fell rapidly to normal within one week in both patients, despite the fact that one was oliguric for three days. The bilirubin was only moderately elevated and the cephalin flocculation fell to 1 plus by discharge. Both patients recovered. These observations led to the present experimental study.

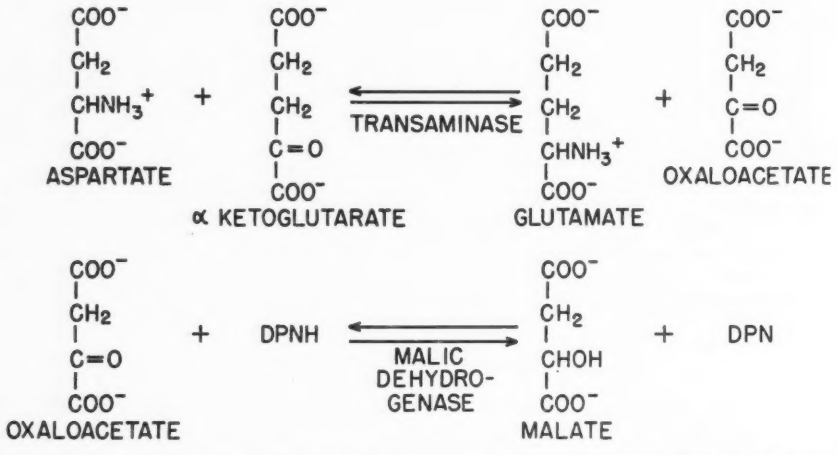

Fig. 1.—Spectrophotometric transaminase assay. A double enzyme system is involved in determining transaminase activity. Serum is added to a substrate containing aspartate and alpha-ketoglutarate, which, in the presence of malic dehydrogenase, oxidizes $DPNH_2$ to DPN. The decrease in optical density is measured in a Beckman spectrophotometer. The micromolar extinction coefficient of $DPNH_2$ is 2.05.

EXPERIMENTAL METHOD

White rats of the Wistar strain weighing 200 to 300 grams, of equal sex distribution, were maintained on a Purina fox chow diet. Initial SGO-T activity was determined on fasting blood secured by cardiac puncture using a No. 25 hypodermic needle under light ether anesthesia. The serum glutamic oxalacetic transaminase activity was periodically measured as well as the serum cholinesterase[3] and alkaline phosphatase activity.[4] Control animals were treated exactly as the experimental animals, except that saline was given by tube rather than carbon tetrachloride. On alternate days, to a comparable group of rats, CCl_4 was administered in 0.1 ml. per kilogram doses via a small soft rubber catheter passed into the stomach.

Measurement of the Enzyme.—The SGO-T activity of the serum was determined spectrophotometrically.[5] The reactant depends upon a double enzyme system with all substrates present in excess (Fig. 1). The decrease in optical density resulting from oxidation of $DPNH_2$ (coenzyme I) to DPN is measured spectrophotometrically. The rate of the reaction is dependent upon the concentration of SGO-T. One unit is designated as a change in optical density of 0.001 per milliliter per minute at wave length 340 mμ. The normal range for adult human beings is 8 to 40 units per milliliter per minute (mean 22.1 with a standard deviation of 6.8); in the rat the normal varies between 170 and 350 units. GO-T activity has been demonstrated in all human and animal sera tested in our laboratory. The return of the serum glutamic oxalacetic transaminase activity to normal levels was followed after four to six intubations of CCl_4. All transaminase determinations were carried out using 0.2 ml. of serum except where greatly increased enzyme activity made the use of a 1:10 dilution of serum necessary. All analyses were made at room temperature (24° C. to 30° C.).

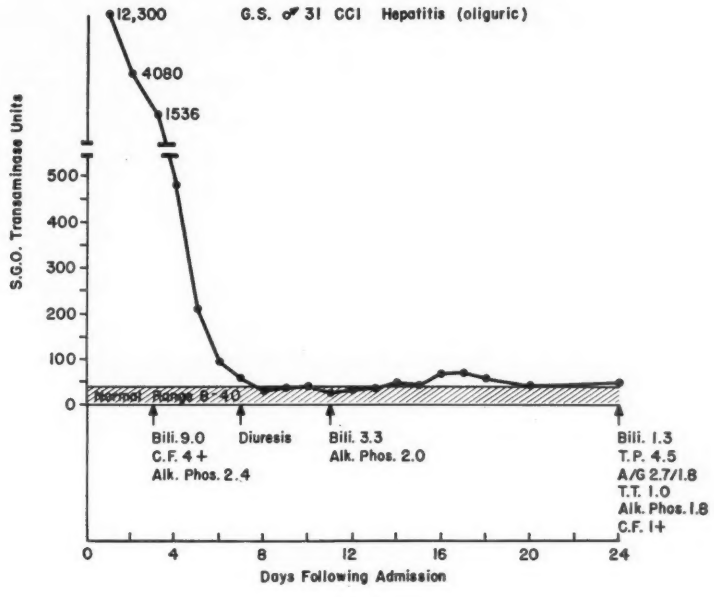

Fig. 2.

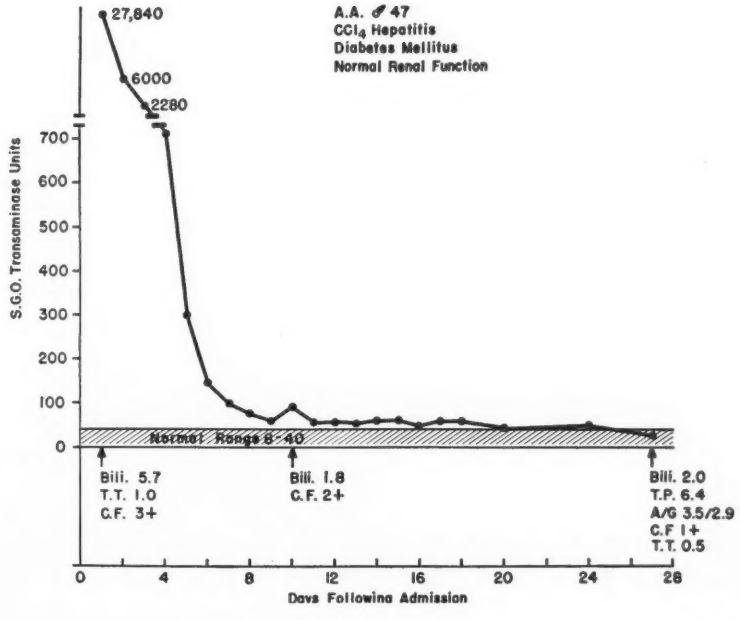

Fig. 3.

Figs. 2 and 3.—Striking elevation of transaminase activity was observed in two patients exposed to CCl_4 two days before hospitalization and the return of the enzyme level to normal as the acute necrotizing process subsided. Moderate bilirubin elevation and positive cephalin cholesterol flocculation tests accompanied the increase of SGO-T activity.

TABLE I. SERUM GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY (UNITS/ML.)*

Carbon Tetrachloride Admin. (0.1 ml./kg.)		NO. RATS	TIME IN DAYS 0	2	4	6	8	10	12	14	16	18	20	24	28
			172		230		200		200			200			
			340		350		330		—			350			
	0.0	Controls 4	250		240		260	220	240	200	215	240	200	220	
			180		200		200	185	220	220	180	200	210	215	
			248		370	220									
			234		340										
	0.4	5	246		360										
			200		400										
			220		480	300	200								
			185				700	420	220						
			—				800								
	0.8	4	—				860	320	200						
			240				900								
			180						1,620	900	300	—	—	200	
			—						2,020	800	—	360	—	240	
	1.2	4	—						3,000	1,200	—	400	ex-pired		
			220						4,350	—	500	330	—	260	220

Av. animal wt. 200 gm.

*The changes observed in transaminase activity in rats weighing 200 grams receiving 0.1 ml. per kilogram carbon tetrachloride by intubation on alternate days are listed. Four animals served as controls. Five animals received a total of 0.4 ml. CCl_4 in two intubations. Their serum transaminase activity returned to normal in two to four days. In four animals receiving 0.8 ml. the SGO-T activity varied from 700 to 900 units per milliliter and returned to normal within four days. In a group of 4 rats receiving 1.2 ml. CCl_4 enzyme activity as high as 4,350 units per milliliter was observed and it required twelve days for the SGO-T to return to normal. No significant change was seen in the SGO-T activity of the control animals.

RESULTS

Microscopic as well as gross study of the liver and kidneys was made in all animals at varying periods. In 69 determinations in normal rats, repeated cardiac puncture using a No. 25 hypodermic needle did not seem to increase transaminase activity. Serum oxalacetic transaminase activity ranged from 100 to 350 units with a mean of 230 units. In the group of animals weighing 200 Gm. after two intubations of CCl_4 the serum glutamic oxalacetic transaminase activity ranged from 370 units per milliliter to 480 units per milliliter (Table I). In several animals, the enzyme activity returned to normal within two to four days following the last intubation of hepatotoxin (Table I, Fig. 4).

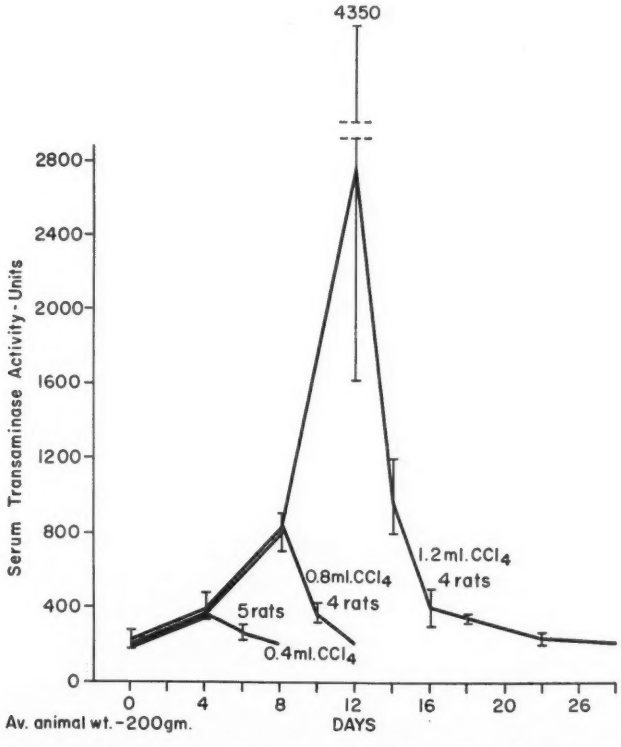

Fig. 4.—The average serum glutamic oxalacetic transaminase activity following administration of different amounts of carbon tetrachloride is shown, the vertical lines indicating the range. With increasing amounts of CCl_4 intubated in divided doses, higher transaminase levels are observed. In the group of animals receiving 1.2 ml. CCl_4, normal levels were observed twelve days following the peak of enzyme activity.

Of this same group, 4 animals received 0.8 ml. CCl_4 (0.1. ml. per kilogram) and their SGO-T activities ranged from 700 to 900 units per milliliter (Table I). In two animals followed without further administration of CCl_4, normal levels were observed in two to four days.

In four rats that received a total dose of 1.2 ml. CCl_4 the activity varied from 1,620 to 4,350 units per milliliter. Three of these animals were serially followed and in 2 of these the transaminase activity was again normal within twelve days following the last dose of hepatotoxin (Table I, Fig. 4).

TABLE II. SERUM GLUTAMIC OXALACETIC TRANSAMINASE ACTIVITY (UNITS/ML.)*

Carbon Tetrachloride Admin. (0.1 ml./kg.)	NO. RATS	TIME IN DAYS 0	2	4	6	8	10	12	14	16	18	20	24	28
0.0 Controls	4	180		185	180									
		184		195	200	210	expired							
		200		180	210	240	200	210	200	220	—	240	200	
		220		210	200	200	185	—	180	—	—	200	210	
		230		800	440	210								
		250		500	expired									
0.6	4	175		700	240	240								
		215		600	—	—								
		—				2,400	700	400	320	—	240			
		—				2,040	1,100	—	520	—	220			
1.2	4	240				4,750	1,600	1,200	700	—	400	—	220	
		165				1,640	1,200	840	600	—	320	—	200	

Av. animal wt. 300 gm.

*Levels of transaminase activity in rats weighing 300 Gm. receiving various amounts of CCl_4 by stomach tube (0.1 ml. per kilogram) are shown. Four control animals show that repeated cardiac punctures by a hypodermic needle do not change the enzyme level. When 0.6 ml. of CCl_4 is administered in two divided doses, the serum transaminase level varied from 500 to 800 units per milliliter, and returned to normal in 6 days. With a larger dose of 1.2 milliliters given in 3 intubations on alternate days, levels from 1,640 to 4,750 units were observed with a return to normal in sixteen days.

Inspection of Fig. 4 reveals that with increasing amounts of CCl_4, higher serum transaminase activities are found. With increasing levels of activity, a longer time for the activity to return to normal is required.

In the group of heavier animals, higher transaminase levels are shown to be reached earlier; this is to be expected from the larger dose of hepatotoxin administered. The SGO-T ranged from 500 to 800 units per milliliter following 0.6 ml. CCl_4 administered in 2 intubations of 0.3 ml. each (0.1 ml. per kilogram) as shown in Fig. 5. In two animals, the enzyme activity had returned to normal 2 and 4 days after the last CCl_4 administration (Table II, Fig. 5).

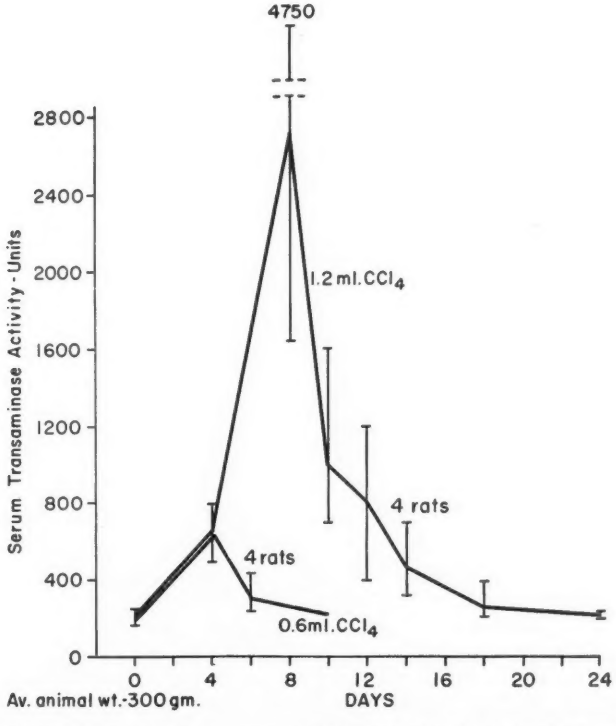

Fig. 5.—Comparison of the average SGO-T activity in animals given a total of 0.6 ml. and 1.2 ml. CCl_4 in divided doses. Rats given 0.6 ml. had peak levels of 500 to 800 units per milliliter while in those given 1.2 ml. CCl_4 the SGO-T rose to 1,640 to 4,750 units per milliliter. In the former, SGO-T fell to normal in six days, but in the latter sixteen days elapsed before SGO-T fell to normal.

When 1.2 ml. of CCl_4 was administered in 4 divided doses the enzyme activity ranged from 1,640 units per milliliter to 4,750 units per milliliter (Table II, Fig. 5). Fig. 5 demonstrates that with increased hepatocellular damage higher enzyme levels are reached. By the end of sixteen days, the transaminase activity had returned to normal. This is in contrast to the previous group where by the end of twelve days the SGO-T activity had returned to normal.

Comparison of result of serum cholinesterase, alkaline phosphatase, and transaminase activities following administration of varying amounts of carbon tetrachloride is illustrated in Fig. 6.

Serum cholinesterase activity is decreased from a mean of 0.18 pH units to 0.12 pH units, the extreme of the lower ranges varying from 0.14 pH units to 0.06 pH units. This represents a 30 per cent to slightly over 50 per cent decline from normal. The alkaline phosphatase activity increased from a mean of 0.18 SJR units to a mean of 40 SJR units representing a 220 per cent increase. The wide range of values encountered in this determination raises the question of the significance of any single determination as a means of evaluating liver function in this particular strain of rats.

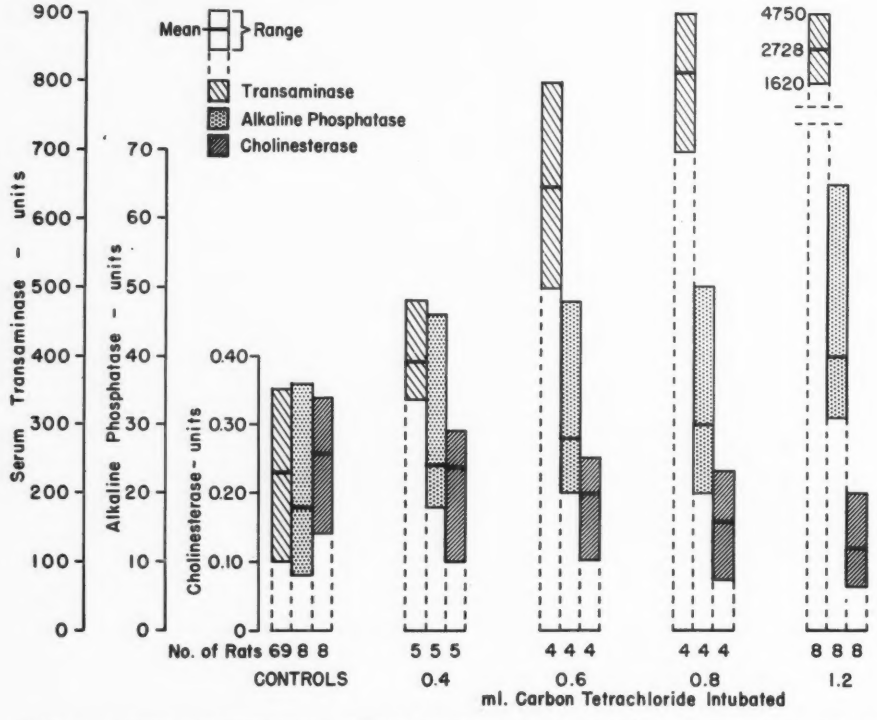

Fig. 6.—Serum transaminase activity compared with alkaline phosphatase and serum cholinesterase in four groups of rats receiving varying total amounts of CCl_4 by intubation in divided doses. The heavy horizontal line in each column is the mean value. The SGO-T activity increased over tenfold (1,000 per cent), the alkaline phosphatase over 100 per cent, and serum cholinesterase, activity decreased 100 per cent.

The serum transaminase activity varied from a mean of 230 units in the control group to a mean of 2,728 units in a group of animals receiving a total dose of 1.2 ml. of CCl_4 during 4 to 6 intubations. This represents a mean increase of over 1,000 per cent in enzyme activity, a far greater change than noted in determination of either the serum cholinesterase or alkaline phosphatase activity. Thus it would seem that in the present experiment, changes in the serum glutamic oxalacetic transaminase activity reflected the degree of hepatocellular damage more closely than changes observed in the determination of the serum cholinesterase or alkaline phosphatase activities.

Pathology.—Grossly the livers of the rats receiving less than 1.2 ml. of CCl_4 showed little change. Two of 6 rat livers had suggestive change consisting of

a very slight yellowish mottled appearance. Gross enlargement was not evident. Microscopic examination, however, revealed slight centrilobular zonal necrosis typical of CCl_4.

With doses of 1.2 ml. CCl_4, 6 of 8 rats had gross evidence of liver damage consisting of a mottled yellowish liver with some apparent enlargement and engorgement. The liver was softer than usual and cut sections showed a nutmeg appearance. Microscopic examinations* revealed moderately severe centrilobular necrosis with early evidence of regeneration (hemotoxylin and eosin stain). In animals with higher SGO-T levels there appeared to be more extensive centrilobular necrosis.

The myocardium and kidneys showed no evidence of gross or microscopic damage with the dosages employed.

CONCLUSIONS

1. Serum glutamic oxalacetic transaminase activity appears to be a highly specific index of hepatocellular injury.

2. With increasing degrees of hepatocellular damage produced by gastric intubation of CCl_4, higher serum transaminase activities are noted. The height and duration of increased SGO-T activity was proportional to the amount of CCl_4 administered and as well as to the severity of liver-cell damage.

3. There appeared to be a correlation between the extent of centrilobular zonal necrosis and the height of SGO-T levels attained. In animals receiving the largest amounts of CCl_4, extensive microscopic necrosis of the liver parenchyma was present. The highest activity was seen in the animals with the most severely damaged livers whether judged by the gross or microscopic picture.

4. Neither the serum cholinesterase nor alkaline phosphatase seems to have the same degree of sensitivity that the serum glutamic oxalacetic transaminase activity possesses in reflecting hepatocellular injury.

5. Serum glutamic oxalacetic transaminase may be a useful guide in following the progress of restoration of hepatocellular integrity following acute injury.

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*By Dr. George K. Higgins.

THE PROTEIN-SPARING EFFECT OF CARBOHYDRATE WITH AND WITHOUT TESTOSTERONE

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WASHINGTON, D. C.

INTRODUCTION

THE addition of carbohydrate to a maintenance diet results in the sparing of protein. Normally nourished men or animals on maintenance diets who are given extra carbohydrate for three to seven days retain variable amounts of nitrogen, from 0.4 to 6.4 mg. of nitrogen for each added carbohydrate calorie.[1-9] The duration of the protein-sparing action of carbohydrate is uncertain. In rats a decrease has been noted in protein sparing after a few days of extra carbohydrate feeding,[3, 9, 10] but this has not been reported to occur in man.

Sparing of protein can also be produced by the administration of testosterone; the literature describing this effect is reviewed in a recent study on the use of testosterone in malnutrition after trauma.[11] Whether or not the protein-sparing effects of carbohydrate and of testosterone are additive is unknown, although Kenyon[12] recorded the failure of testosterone to spare protein in a single patient on a very high carbohydrate intake. Some attention has been given to the possible influences of other hormones on the nitrogen sparing effect of extra carbohydrate. Following pancreatectomy,[13, 14] hypophysectomy,[15] or thyroidectomy,[16] protein sparing by carbohydrate is unaffected.

The present study deals with the magnitude and duration of the protein-sparing effect of carbohydrate added to a maintenance diet in three normally nourished men. In addition, an attempt was made to increase the effect by the administration of testosterone.

METHODS

Three patient volunteers were studied by the nitrogen balance technique. Case summaries are recorded following. Patient P. S. suffered from cirrhosis of the liver, patient T. K. from Reiter's syndrome, and patient H. H. from rheumatoid spondylitis. All subjects were restricted to a metabolic ward but were ambulatory throughout the experiments. Urine collections were made for twenty-four-hour periods and refrigerated at 8° C. without added preservative. Stool collections were obtained over six- to eight-day periods with the specimen constantly refrigerated at 8° C.

The patients received basal diets of constant composition throughout the periods of study. P. S. and T. K. received solid diets which were made up in advance for the entire study period and stored in the Deepfreeze until prepared for the patients. Patient H. H. was given a liquid diet prepared fresh daily. At least four half-diet aliquots of each diet were analyzed for nitrogen, sodium, and potassium; the analyzed compositions checked within

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**Major, MC, USA.

5 per cent. Averages of all analyses were used in calculating the patients' intakes. The carbohydrate supplement consisted of hard candy, Coca-Cola, corn syrup, or lactose. The supplement was consumed between meals, the day's intake always being completed within four hours after supper.

The macro-Kjeldahl method was used to determine the nitrogen content of diet, urine, and stool. Urine collections examined qualitatively for sugar through each study showed no glycosuria. Body weights were determined once daily after voiding, before breakfast, on a scale accurate to ± 25 Gm.

CASE REPORT

P. S., a 58-year-old man, height 64 inches, had symptoms and signs of chronic liver disease dating back to October, 1948, when hepatomegaly and abnormal liver function tests were found. This man had imbibed heavily of beer and wine in the past and at one time was addicted to heroin. Observation from October, 1948 to February, 1952, showed a gradual worsening of liver function. In May, 1952, needle biopsy of the liver revealed marked portal fibrosis and histologic findings compatible with Laennec's cirrhosis. Ascites first appeared in September, 1952, and progressed until admission to the hospital in November, 1952. At that time ascites, peripheral edema, hepatomegaly, and numerous spider angiomata were evident. Weight on Nov. 22, 1952, was 79.8 kilograms. At the time the present study was begun in February, 1953, the patient appeared moderately obese. Although ascites and edema could no longer be detected, the patient lost 2 kilograms of weight accompanied by a diuresis of sodium during the first ten days of the study. The weight at that time was 72.5 kilograms. The liver was palpated 8 cm. below the right costal margin on inspiration. Bromsulphalein retention was 37 per cent in forty-five minutes; total bilirubin 0.94 mg. per cent; prompt direct bilirubin 0.25 mg. per cent; thymol turbidity 6.1 units; zinc turbidity 14.9 units; cephalin cholesterol flocculation 3+; total protein 9.75 Gm. per cent, with albumin 3.06 Gm. per cent and globulin 6.69 Gm. per cent. At the completion of the study on March 10, 1953, there had been no change in the clinical findings. Bromsulphalein retention was 33 per cent in forty-five minutes; total bilirubin 0.75 mg. per cent; prompt direct bilirubin 0.23 mg. per cent; thymol turbidity 6.5 units; zinc turbidity 14.9 units; cephalin cholesterol flocculation 3+; total protein 9.21 Gm. per cent, with albumin 2.92 Gm. per cent and globulin 6.29 Gm. per cent.

T. K., a 24-year-old man, height 73 inches, had recurrent episodes of arthritis of the knees, ankles, and hands, urethritis, and conjunctivitis beginning in November, 1951, diagnosed as Reiter's syndrome. His last exacerbation of symptoms began in December, 1952, and cleared with phenylbutazone therapy. In March, 1953, therapy with aurothioglucose, 25 mg. per week, was begun and continued throughout the study period in May and June, 1953. During the period of study there were no signs of activity of the patient's illness, and sedimentation rates were consistently normal. The patient's weight had been stable for eighteen months prior to the beginning of the balance program. At the start of study, the patient weighed 74.5 kilograms and appeared well nourished.

H. H., a 23-year-old man, height 71 inches, developed low back pain in August, 1952, followed over the subsequent few weeks by pain on motion of the left shoulder and left hip and progressive stiffness of the spine. The patient continued his duties, however, until August, 1953, when pain and stiffness of the spine caused him to seek medical care. Examination on Aug. 27, 1953 showed marked limitation of all motions of the thoracic and lumbar spine, with tenderness over the erector spinae muscles and the vertebral spinous processes. Roentgenograms of the thoracic and lumbar spine showed slight demineralization of the vertebral bodies and haziness of the apophyseal joints. The sacroiliac joint margins were indistinct and surrounding bone was sclerotic. Sedimentation rate ranged from 10 to 17 mm. per hour. The patient was believed to have rheumatoid spondylitis. The present study was carried out at a time when the patient's only treatment was physiotherapy. At the beginning of the study in November, 1953, the patient weighed 71.0 kilograms and appeared well nourished.

RESULTS

Table I summarizes the experimental conditions and results.

Although patient P. S. received a minimum calorie and protein intake during Period 1, the diet was sufficient to maintain positive nitrogen balance; the weight loss was accompanied by sodium diuresis and is believed to represent loss of clinically imperceptible ascites and edema (Fig. 1). Significant further nitrogen retention occurred with testosterone in Period 2, when compared with Periods 1 and 3 (Table II). The carbohydrate supplement produced marked increased nitrogen retention which persisted unchanged for the entire eighteen-day period of supplementation. When testosterone was added at the higher calorie intake, no further nitrogen sparing took place.

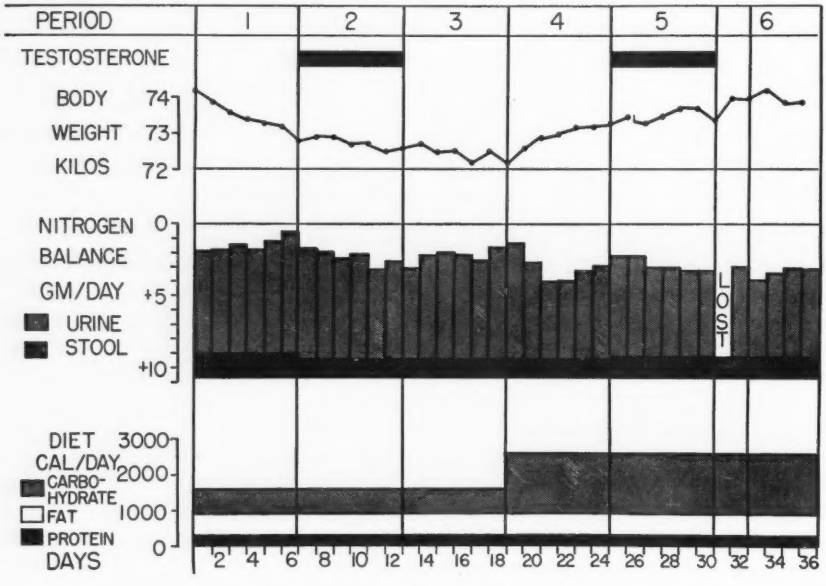

Fig. 1.—Balance data of Subject P. S.

The carbohydrate supplement produced an average increase of nitrogen retention of 1.7 Gm. per day in Patient T. K., which remained undiminished after fifteen days (Fig. 2). Testosterone administration did not cause significant additional nitrogen sparing beyond that from carbohydrate. With later return to the basal diet, although the nitrogen balance had changed in Period 5 as compared with Period 1, testosterone now caused significant nitrogen retention.

Although Patient H.H.'s weight was stable, he did not maintain nitrogen equilibrium on the basal diet (Fig. 3). This loss of nitrogen may be a manifestation of his rheumatoid spondylitis. Testosterone produced significant nitrogen retention when the patient was taking the basal diet, when Period 2 is compared with Periods 1 and 3. When testosterone and the carbohydrate supplement were given together for twelve days, significant nitrogen retention was maintained. The degree of retention was similar to that produced by carbohydrate alone in the first two patients.

TABLE I. SUMMARY OF EXPERIMENTAL CONDITIONS AND RESULTS

PATIENT	PERIOD	SUPPLEMENT*	CAL./KG.	PROTEIN GM./KG.	DAYS†	MEAN NITROGEN BALANCE (GM.)
P. S.	1		21	0.7	4	+1.2
	2	T.P.	21	0.7	4	+2.6
	3		21	0.7	4	+2.1
	4	CHO	35	0.7	4	+3.5
	5	CHO, T.P.	35	0.7	4	+3.1
	6	CHO	35	0.7	4	+3.3
T. K.	1		40	1.1	4	+1.3
	2	CHO	54	1.1	6	+2.3
	3	CHO, T.P.	54	1.1	2	+2.9
	4	CHO	54	1.1	1	+2.0
	5		40	1.1	2	−0.8
	6	T.P.	40	1.1	3	+1.4
H. H.	1		41	1.5	5	−0.2
	2‡	T.P.	41	1.5	3	+1.0
	3		41	1.5	6	−0.9
	4§	CHO, T.P.	56	1.5	8	+1.6

*T.P.: Testosterone propionate, 25 mg. 1 M. daily; CHO: 1,000 calories carbohydrate.
†First two days of each period omitted from calculations.
‡Day 5 of period omitted from calculations.
§Period includes two 48-hour urine collections.

TABLE II. STATISTICAL EVALUATION OF EXPERIMENTAL RESULTS

FACTOR TESTED		SUBJECT	PERIODS COMPARED	DIFFERENCE IN MEAN NITROGEN BALANCE	POOLED STANDARD ERROR*	PROBABILITY
Identity: Basal Diet, before and after testosterone		P. S.	1 vs. 3	+0.9	0.33	<0.05
		T. K.	1 vs. 5	−2.1	0.36	<0.01
		H. H.	1 vs. 3	−0.7	0.68	Not sig.
Identity: High CHO diet, before and after testosterone		P. S.	4 vs. 6	−0.2	0.33	Not sig.
		T. K.	2 vs. 4	−0.3	0.36	Not sig.
Effect of high carbohydrate diet	A.	P. S.	3 vs. 4	+1.4	0.33	<0.01
		T. K.	1 vs. 2	+1.0	0.29	<0.01
	B.	P. S.	1, 3 vs. 4, 6	+1.7	0.27	<0.01
		T. K.	1, 5 vs. 2, 4	+1.7	0.47	<0.01
Effect of testosterone, on basal diet	A.	P. S.	1 vs. 2	+1.4	0.36	<0.01
		T. K.	5 vs. 6	+2.2	0.67	<0.05
		H. H.	1 vs. 2	+1.2	0.76	Not sig.
	B.	P. S.	1, 3 vs. 2	+0.9	0.38	<0.05
		T. K.	1, 5 vs. 6	+0.9	0.91	Not sig.
		H. H.	1, 3 vs. 2	+1.6	0.63	<0.05
Effect of testosterone, on high carbohydrate diet	A.	P. S.	4 vs. 5	−0.4	0.27	Not sig.
		T. K.	2 vs. 3	+0.6	0.34	Not sig.
	B.	P. S.	4, 6 vs. 5	−0.3	0.23	Not sig.
		T. K.	2, 4 vs. 3	+0.6	0.32	Not sig.
Effect of testosterone and high carbohydrate diet		P. S.	1, 3 vs. 5	+2.8	0.32	<0.01
		T. K.	1, 5 vs. 3	+2.3	0.92	<0.05
		H. H.	1, 3 vs. 4	+2.2	0.44	<0.01

*Pooled standard error[24] $= \dfrac{\sqrt{\dfrac{\Sigma(x_1 - m_1)^2 + \Sigma(x_2 - m_2)^2}{n_1 + n_2 - 2}}}{\sqrt{\dfrac{n_1 n_2}{n_1 + n_2}}}$

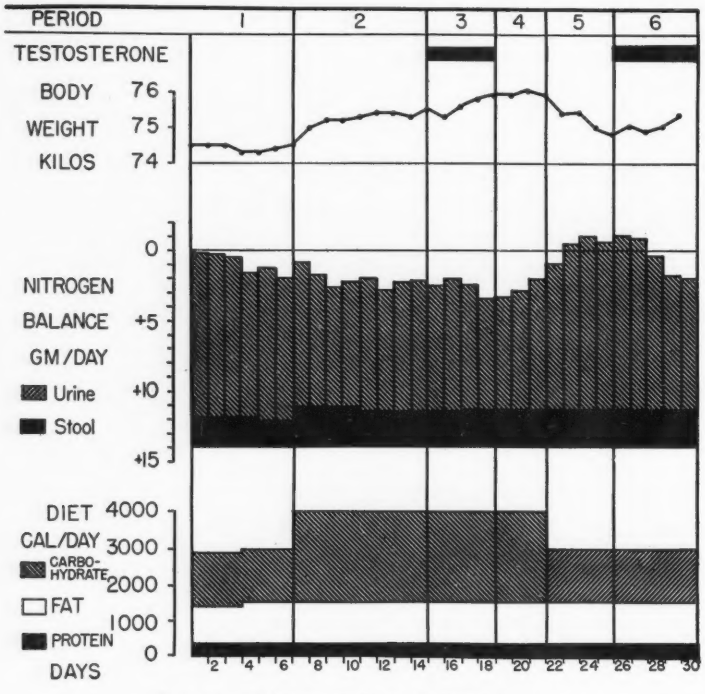

Fig. 2.—Balance data of Subject T. K.

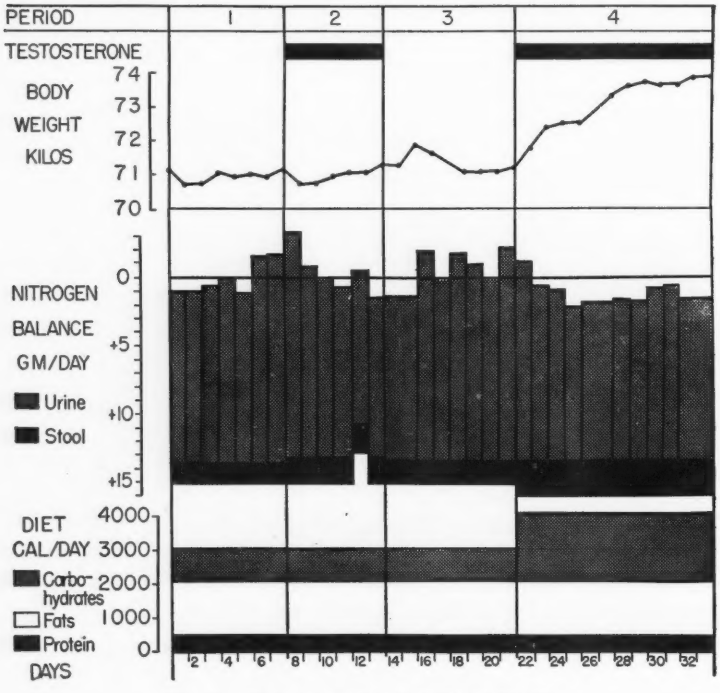

Fig. 3.—Balance data of Subject H. H.

In Table II are listed the average differences in nitrogen balance produced in these experiments, together with the results of statistical evaluation. Significant and comparable nitrogen sparing occurred with either testosterone or carbohydrate alone. During carbohydrate feeding no significant extra retention was produced by testosterone administration. The average increase in nitrogen retention brought about by carbohydrate and testosterone together was, however, very slightly greater than that brought about by either supplement alone.

The nitrogen retention produced by 25 mg. of testosterone per day ranged from 12 to 30 mg. per kilogram of body weight. The nitrogen spared by carbohydrate varied from 1.0 to 1.7 mg. per added calorie. All three patients gained weight during the periods of carbohydrate supplementation. The average gains were: P. S., 90 Gm. per day; T. K., 100 Gm. per day; and H. H., 220 Gm. per day. Testosterone caused no gains in weight, nor did it produce clinical edema; it produced no sodium retention in patient P. S. in whom sodium balances were measured.

DISCUSSION

In the present study the addition of carbohydrate to a maintenance diet led to sparing of protein for as long as the supplement was given. Subjects P. S. and T. K. retained, respectively, 1.4 and 1.7 mg. nitrogen for each added calorie. Other observations in well-nourished human beings[1, 2, 8] have shown retention of 0.9 to 1.8 mg. nitrogen per carbohydrate calorie added to a maintenance diet. This similarity is notable in that the carbohydrate supplement in the present study was given not at mealtime, but for a period of two to four hours after meals. Larson and Chaikoff[4] orginally observed, and it has been amply confirmed,[15, 17-19] that the carbohydrate of a maintenance diet was most effective in sparing protein when given with the rest of the diet. Carbohydrate given two hours after consumption of the diet was about one-half as effective in sparing protein, while that given four hours after the diet had no protein-sparing effect. Cuthbertson and Munro[17] subsequently pointed out that no such time limit is applicable to carbohydrate fed in addition to a maintenance diet. Their belief that extra carbohydrate continues to spare protein as effectively four hours after a meal as it does with a meal receives support from the present study.

The mechanism of protein sparing by carbohydrate is unknown. Suggestions that ". . . carbohydrate spares protein by relieving it of the necessity of providing carbohydrate . . .[20] or that carbohydrate may contribute to the production of amino acids[21] have been made. Munro[22] believed that the gain in high energy phosphate bonds resulting from metabolism of the extra carbohydrate contributed to the formation of peptides. Consideration of the ratio of extra nitrogen gained and body weight gain during the period of carbohydrate supplementation in the present study suggests that "obesity tissue" is being formed as a result of the added calorie intake. Keys and Brozek[23] estimated obesity tissue to contain 1.1 per cent nitrogen, presumably in the supporting structure. Lean tissue contains 3 to 5 per cent nitrogen. In Subject P. S. 1.5

per cent of the weight gained during carbohydrate supplementation is accounted for by the extra nitrogen retention. In Subject T. K. the figure is 1.7 per cent, and in Subject H. H., who received testosterone at the same time, nitrogen accounts for 1.0 per cent of the weight gained.

The ability of testosterone to induce nitrogen retention is apparent in the periods of its use during the basal diet in all three subjects. The amount of nitrogen retained appears to be slightly less for the same dose than that found in malnourished patients,[11] 12 to 30, as compared with 16 to 56 mg. nitrogen per kilogram. During the periods of high carbohydrate feeding, however, testosterone caused no significant additional nitrogen retention in Subjects P. S. or T. K. In Subject H. H., who received testosterone and the carbohydrate supplement together, the degree of nitrogen retention was less than that expected if the protein-sparing effects of these two are additive. The carbohydrate supplement, however, may not have inhibited the effect of testosterone completely; the average increases in nitrogen retention during periods of administration of both carbohydrate and testosterone were greater in all three subjects than the increases caused by either separately, although these changes were not significant (Table II). In malnourished patients, calorie intake appeared to have no influence on the effect of testosterone.[11] The blocking effect here reported may, therefore, be peculiar to carbohydrate, or may occur only in well-nourished individuals.

Since the mechanisms are unknown by which carbohydrate and testosterone conserve nitrogen, no explanation of the effects observed is at hand. The fact, demonstrated here, that extra carbohydrate blocks the protein-sparing effect of testosterone suggests that both supplements may act through the same mechanism. If the action of surfeit carbohydrate is simply the formation of obesity tissue, as suggested previously, it might prove fruitful to study the effect of testosterone on the synthesis of fat.

SUMMARY

1. The protein-sparing effect of carbohydrate added to a maintenance diet continued undiminished for fifteen, sixteen, and eighteen days in the subjects studied.

2. The ability of testosterone to cause nitrogen retention was diminished or abolished during periods of high carbohydrate feeding.

We wish to thank Dr. Victor M. Sborov for his helpful suggestions and advice; Captain Esther Rosenberg and 1st Lt. Margaret Shipman and staff for their careful supervision of the diets; and Marjorie Knowlton, William Sharon, Margaret Bollier, Betty Hackley, Joseph Bruton, and Edward Tracy for their careful analytical work.

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NONDIALYZABLE MATERIAL IN NORMAL HUMAN URINE*

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INTRODUCTION

STUDIES on the chemical nature of high molecular weight material in normal human urine have been generally confined to fractions of the total material. Analytic data on fractions obtained by methods of precipitation[1-8] have shown that much of this material consists of a mixture of carbohydrate and protein. Some of this carbohydrate material exhibits type specific blood group properties.[9-11] Tamm and Horsfall[12] have obtained a homogeneous mucoprotein from urine by NaCl precipitation. Other workers, using methods of ultrafiltration[13, 14] have obtained viscous urine colloids. Electrophoretic studies were made on that portion of the total colloids soluble in saline. Mobilities closely resembling those of albumin and several globulin components of plasma were demonstrated. Further analysis of the insoluble portion was not described. This residue may contain the Tamm and Horsfall mucoprotein.

After thorough dialysis, urine contains all the high molecular weight carbohydrate and protein material as well as other substances which fail to pass through the dialyzing membrane. It seemed worth while to obtain this total material and conduct a number of studies upon it. A dialyzed concentrate of urine was lyophilized and a product obtained which has been termed nondialyzable urine solids or, more simply, urine solids. The results of chemical and chromatographic investigations on this material are presented in this paper.

MATERIALS AND EXPERIMENTAL METHODS

1. *Preparation of Nondialyzable Urine Solids.*—Normal male subjects collected the total urinary output during a 24-hour period. The urine was refrigerated during the collection time. Thymol was added as a preservative. The total volume (usually about 700 ml.) was dialyzed for three days against running tap water at 10° C. After dialysis the urine was concentrated at 30° to 40° C. in vacuo to about 200 ml. and this concentrate was again dialyzed for three days at 5° C. against twice-daily changes of distilled water. In some samples a very small sediment was removed by centrifugation. Further concentration in vacuo was carried out until about 30 ml. of a dark, syrupy liquid remained. This was lyophilized. Samples of this material derived from different subjects were designated Ph, Re, Ba, and De-Sh (pool). The yields ranged from 0.150 to 0.400 Gm. per liter of urine. About 8 to 12 mg. of the solid sample was added to a 10 ml. volumetric flask and dissolved in 0.08 N NaOH. Aliquots of this solution were used for fucose, hexuronic acid, and nitrogen determinations. Portions of the solid material were hydrolyzed with 2 ml. 4 N HCl in sealed tubes at 100° C. for 6½ hours and diluted to 25 ml. with distilled water; hexosamine and hexose analyses were made on aliquots of this hydrolysate.

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*The opinions expressed in this article are those of the authors and do not necessarily represent those of any government agency.

2. *Chromatographic Methods.*—

a. *Hydrolyzed material:* About 20 mg. of urine solids was hydrolyzed for two hours in a sealed tube with 1 ml. of 2 N H_2SO_4.[15] Twenty to 30 mg. of acidic mucopolysaccharides (heparin, chondroitin sulfate, and hyaluronate) and 1 ml. of dialyzed, concentrated blood group substances A and B from hog and horse stomach* were similarly hydrolyzed. The pH of the hydrolysate was brought to 5 with solid $Ba(OH)_2$ and the $BaSO_4$ removed by centrifugation.

Ascending chromatography was used exclusively and methods are described in detail elsewhere.[16] Two unidimensional runs were made in 1-butanol, pyridine, and water (3:2:1.5). However, this technique failed to separate xylose and fucose, and mannose, fructose, and arabinose. To achieve these separations phenol saturated with water was used for chromatography in the second dimension. Forty to 70 λ of the hydrolysates were applied to the paper. Standard sugars and hexuronic acids were run for comparison. A β-naphthylamine spray was used. Hexosamines were demonstrated on separate chromatograms by successive spraying with acetylacetone and Ehrlich's reagent (p-dimethylamino-benzaldehyde).

b. *Nonhydrolyzed material:* Ascending chromatography of urine solids was carried out without preliminary hydrolysis in an attempt to separate and tentatively identify high molecular weight components. Details of the technique are discussed elsewhere.[17] Twenty milligrams of urine solids was dissolved in 1 ml. of distilled water. Ten milligrams of acidic mucopolysaccharides dissolved in 1 ml. of distilled water, or in 1 ml. of 0.01 M NaCl in the case of hyaluronate, was used as standards. Dialyzed, concentrated blood group substances were used as examples of neutral mucoids. About 15 to 30 λ of the solution of urine solids, 1-2 λ of chondroitin sulfate and heparin, 10 λ of hyaluronate, and 30 λ of blood group materials were applied to duplicate chromatograms. Varying proportions of isopropanol mixed with phosphate buffers were used as solvents. The dried chromatograms were fixed in neutral Formalin and ethanol. One chromatogram was sprayed with toluidine blue, the duplicate with mucicarmine.

3. *Analytic Methods.*—A modified Elson-Morgan procedure was used for hexosamine.[18] The anthrone method as described by Scott and Melvin[19] was used for hexose determinations. Optimum heating conditions for color development proved to be 95° C. for a period of eight minutes. Nitrogen was determined by a micro-Kjeldahl method.[20, 21] Protein was determined by a micromethod.[22] The method of Dische and Shettles[23] was followed for fucose analysis. Samples of urine solids in dilute NaOH solution were not hydrolyzed prior to analysis. The naphthoresorcinol method described by Corn Products Sales Company[24] was used for hexuronic acid analysis. A number of time studies showed that preliminary hydrolysis of urine solids was required. Optimum yields were obtained using a one-hour period of hydrolysis at 100° C. More protracted hydrolysis led to destruction and loss of color. Two milliliters of a solution of urine solids in dilute NaOH was hydrolyzed in concentrated C.P. HCl under these conditions in 16 by 150 mm. acid-washed Pyrex test tubes.† Naphthoresorcinol solution was then added to the cooled tubes, as well as to tubes containing water blanks, and glucuronic acid standards. All the tubes were heated at 100° C. for thirty minutes. Ether extractions under ice-cold conditions followed, and the optical density of the ether extracts was determined in stoppered, chilled cuvettes with a Model B Beckman spectrophotometer set at 570 mμ.

Ash was determined by weighing samples of urine solids before and after combustion at 600° C. for fifteen minutes. Total sulfur was determined by the method of Kahane.[25] For analysis of sulfate-sulfur, 40 mg. of urine solids was mixed with 0.1 N $HClO_4$ and kept at 85° C. for twelve hours and at room temperature for four days. The solution was mixed with ethanol, 1 ml. 0.01 M K_2SO_4, 1 ml. 1 M KNO_3, and titrated amperometrically with 0.01 M $PbNO_3$.[26] The amount of $PbNO_3$ required to reach the inflection point in excess of that required for the added K_2SO_4 was determined.

*Sharp & Dohme, Inc.
†Obtained from H. S. Martin Company, Evanston, Ill.

TABLE I. ANALYSES OF NONDIALYZABLE URINE SOLIDS FROM NORMAL HUMAN URINE

SAMPLE	YIELD		PER CENT HEXOSAMINE	PER CENT HEXOSE	PER CENT FUCOSE	PER CENT HEXURONIC ACID	PER CENT NITROGEN	PER CENT PROTEIN	PER CENT ASH	PER CENT TOTAL S	PER CENT SO_4-S
	GM./l URINE	GM./24 HR.									
Ph	0.436	0.305	9.8	15.6	2.4	1.3	9.4	--	--	--	--
Re	0.349	0.336	9.8	14.0	2.6	1.3	10.1	--	--	--	--
Ba	0.151	0.218	10.3	13.7	3.1	1.3	8.6	49.5	8.5	1.4	0.15
De-Sh (pool)	0.173	--	10.8	18.1	3.1	1.8	8.5	--	9.5	1.9	--

Blood group activity was determined by modifying the method of Morgan and van Heyningen.[27] A 0.1 per cent (w/v) solution of urine solids in 0.15 M NaCl was used.

RESULTS

1. *Analytic Data.*—The analytic data on nondialyzable urine solids are presented in Table I. Samples of this material from different subjects showed similar chemical analyses.

Nonhexosamine nitrogen was accounted for as protein-nitrogen by the use of a microprotein method. Protein and carbohydrate components made up about 80 per cent of the weight of nondialyzable solids. A further significant contribution of not quite 10 per cent was made by inorganic ash.

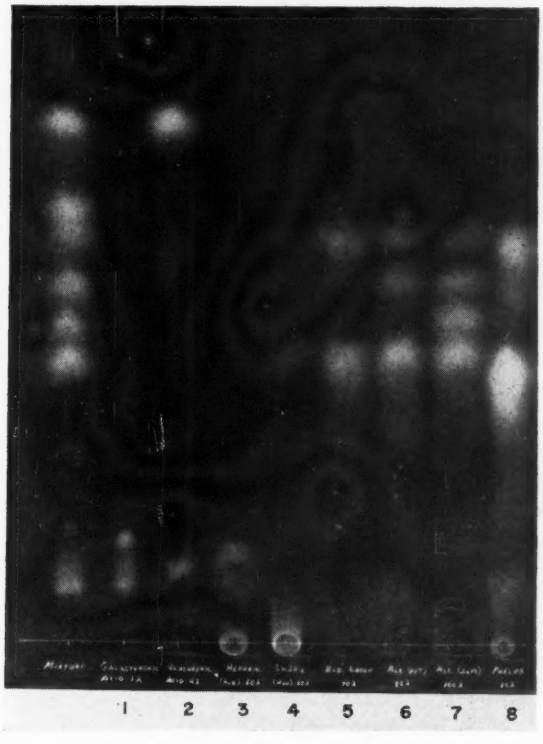

Fig. 1.—Unidimensional ascending chromatogram of *hydrolyzed* urine solids and fractions obtained from urine compared with standard substances. Acidic mucopolysaccharides and blood group substances A and B were similarly hydrolyzed. Mixture, far left, consists of (from the top down): glucuronolactone, ribose, fucose, mannose, glucose, galactose, and glucuronic and galacturonic acids. The latter two substances are not separated. Along the line of origin are: *1*—galacturonic acid; *2*—glucuronic acid and its lactone; *3*—heparin; *4*—chondroitin sulfate; *5*—blood group substances A and B; *6*—the precipitate obtained from urine by addition of ethanol-HCl; *7*—supernatant of *6*; *8*—nondialyzable urine solids (sample Ph.).

2. *Chromatographic Analysis.*—

a. *Simple sugars and hexuronic acid:* Ascending chromatography in one and two dimensions (Figs. 1 and 2) revealed the constant presence of spots corresponding to those of standard fucose, mannose, galactose, and hexuronic acid. Glucose was found in some samples. In the two-dimensional chroma-

togram, a faint, unidentified spot was noted 1 inch above and to the left of the hexuronic acid spot. On the same chromatogram spots corresponding to traces of xylose and ribose were visible immediately after spraying.

Hexosamine was demonstrated on separate chromatograms, but the chromatographic identification of specific amine sugars was not undertaken.

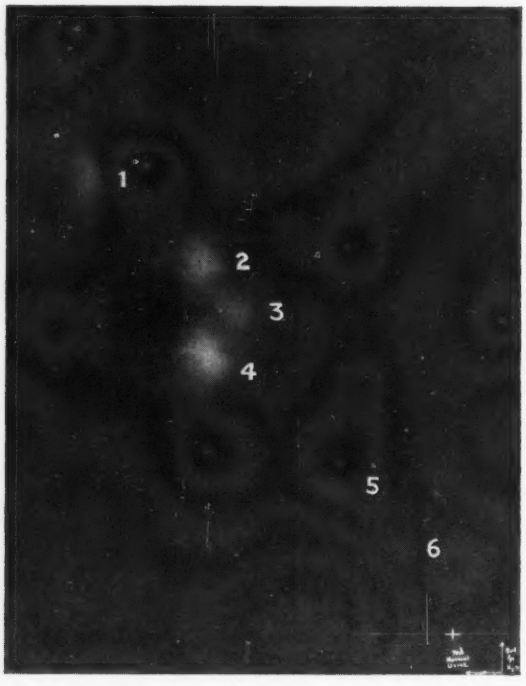

Fig. 2.—Two dimensional ascending chromatogram of *hydrolyzed* urine solids (sample De-Sh). *1*—fucose; *2*—mannose; *3*—glucose; *4*—galactose; *5*—unidentified component; and *6*—hexuronic acid. Visible on the freshly sprayed chromatogram, but not evident in this figure, were spots attributed to ribose and xylose, located between fucose and mannose.

b. *High molecular weight components of urine solids:* Fig. 3 shows a photograph of chromatograms run in duplicate. The paper on the left was sprayed with toluidine blue. Mucopolysaccharides with free acidic groups, such as chondroitin sulfate, heparin, and hyaluronate, stained metachromatically and appeared purple in contrast with the light-blue background of the paper. Neutral substances, or those in which reactive anionic groups are masked, may not stain, or may stain a faint blue, i.e., orthochromatically, and be distinguished with difficulty from the background coloration.

The paper on the right was sprayed with mucicarmine. Neutral carbohydrate material, such as blood group substance, which fails to stain distinctly with toluidine blue, stained pink. Acidic mucopolysaccharides are also stained pink by this dye.

All urine solids tested showed an acidic substance migrating at a rate similar to chondroitin sulfate. Standard protein-free chondroitin sulfate derived from cartilage and chondroitin sulfate containing 14 per cent protein*

*General Biochemicals, Inc.

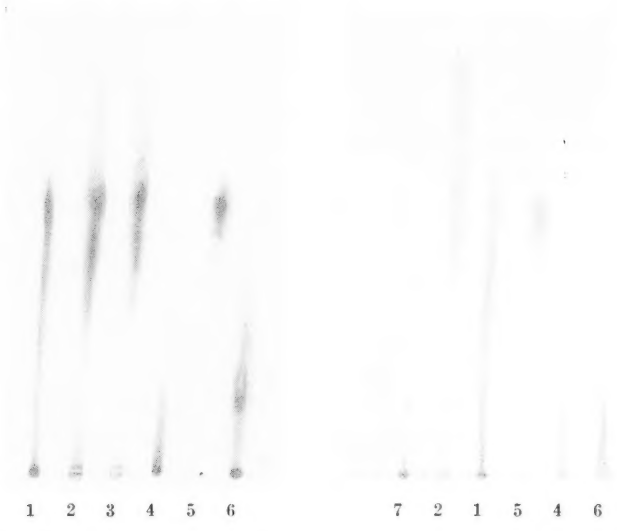

Fig. 3.—Unidimensional ascending chromatograms of *nonhydrolyzed* urine solids, standard acidic mucopolysaccharides and a neutral mucoid. Chromatogram on left sprayed with toluidine blue. *1*—sample De-Sh; *2* and *3*—sample Ph (a greater amount of this sample was applied at *2*); *4*—heparin; *5*—chondroitin sulfate; *6*—hyaluronate. Chromatogram on right sprayed with mucicarmine. *7*—blood group substances A and B. The rest of the numbers are identified above.

showed identical migration. This substance in urine solids did not separate from standard chondroitin sulfate when the two were superimposed in one spot and chromatographed. A second acidic substance was repeatedly noted in the sample Ph (Fig. 3). This component stained metachromatically and migrated more slowly than standard chondroitin sulfate. In this same sample of urine solids there also appeared a faint orthochromatic streak at the top of the paper. A somewhat more discrete spot was noted in the same location on the paper sprayed with mucicarmine. These staining reactions suggested a neutral component of the urine solids, or possibly one without free anionic groups.

3. *Identification of Blood Group Substance.*—Type specific blood group material was demonstrated in low titer in urine solids of Groups A and B subjects. Solutions of urine solids in 0.15 M NaCl inhibited the agglutination of homologous red cells by homologous antiserum. Samples of nondialyzable solids proved serologically less active than fractions obtained from urine by alcohol-acid precipitation.

DISCUSSION

Analytic data presented in this paper were obtained on the total nondialyzable material in normal urine and cannot be compared directly with fractions derived by other workers using methods of precipitation. Furthermore, as would be expected, the yields reported in this paper run considerably higher than the recovery of material obtained by other methods.

Certain components which may be expected to be part of nondialyzable solids were not accounted for in the analytic procedures. These included bound water, acetyl, pigment content, and possibly sialic acid, an acidic component of mucoproteins of biologic origin.[8, 28]

1. *Neutral Sugars.*—The presence of hexose sugars and fucose has been determined by analytic and chromatographic techniques. The tentative nature of chromatographic identification is recognized and preparation of crystalline derivatives is in progress in this laboratory. The following points of evidence support the validity of the findings presented: (a) similar migration of standard sugars and those in hydrolyzed urine solids was confirmed in the following solvent mixtures: 1-butanol, pyridine, and water (3:2:1.5); phenol saturated with water; isoamyl alcohol, pyridine, and water (3.5:3.5:3); 2,6-lutidine, 2,4,6-trimethylpyridine, and water (1:1:2); and isopropanol *or* isobutanol, glacial acetic acid, and water (7:2:1); (b) appearance of only the expected spots on the chromatograms when hydrolysate and known sugars were superimposed and run as a mixture in both one and two dimensions; (c) use of hydrolyzed blood group substances A and B as a standard for identification of fucose, galactose, and hexosamine (Fig. 1). Crystalline derivatives of these sugars have been prepared from Group A substance derived from hog mucosa[29]; (d) distinguishing color characteristics imparted by the β-naphthylamine spray. Pentoses appeared orange-red, aldohexoses brown, and methylpentoses yellow.

Using exclusively unidimensional chromatography, a number of workers [7, 8, 30-32] have found fucose, mannose, galactose, and, occasionally, glucose and xylose in fractions obtained from urine by methods of precipitation.

2. *Hexuronic Acid.*—The presence of hexuronic acid in hydrolysates of nondialyzable urine solids has been demonstrated both by chemical analysis using the naphthoresorcinol method and by chromatography. Characteristically colored pink spots were observed on chromatograms of urine solids after spraying with β-naphthylamine. These spots corresponded in position and color with glucuronic acid derived from hydrolysis of heparin (Fig. 1) and with standard glucuronic and galacturonic acids. Chromatographic separation of these hexuronic acids was not undertaken. The questionable reliability of the chemical methods for determination of hexuronic acid is recognized and the quantitative figures given in Table I for the contents in the nondialyzable urine solid preparations should be considered as estimates.

A hexuronic acid component has been previously reported in chromatographic analysis of fractions obtained from urine only by Tayeau and associates.[32]

3. *Hexosamine.*—The chemical method used for the determination of hexosamine content of the urine solids is supported by extensive previous work.[18, 33, 34] Hexosamine was also demonstrated in the present studies by means of paper chromatography using pure glucosamine as a standard.

4. *High Molecular Weight Components.*—The analytic data indicating the presence of hexuronic acid, sulfate, and neutral sugars are consistent with the chromatographic findings of high molecular weight components, which, by their staining reactions, appear to contain acidic, and, in one sample, neutral carbohydrate moieties. The most constant acidic component migrates with chondroitin sulfate. Kerby[35] has reported a similar finding in the fraction obtained by benzidine precipitation of urine. However, on a chromatographic basis alone, especially without comparison with a larger number of standards, the nature of the acidic component cannot be considered as established.

5. *Carbohydrate and Protein in Urine Solids.*—Chemical analysis of nondialyzable solids from normal urine has shown that the major part of the weight of the solids is contributed by a mixture of carbohydrate and protein material. Kobayasi[1] and we have found that the carbohydrate and protein appear to be bound together in part, since attempts to denature and remove protein with chloroform-amyl alcohol mixtures failed to significantly reduce the nitrogen content. The combination of at least a part of the carbohydrates with protein is also implied by the isolation from urine of a homogeneous mucoprotein by Tamm and Horsfall.[12, 36] The enormous molecular weight of this mucoprotein[36] and its lack of immunologic identity with mucoproteins of plasma,[37] suggest that it is derived from the urinary tract, presumably the bladder.[38] Whether mucoproteins of plasma and connective tissues are present in normal urine in significant amounts and constitute part of the total high molecular weight material cannot be answered at this time.

SUMMARY

Normal human urine has been dialyzed, concentrated at low temperature in vacuo, and lyophilized to obtain nondialyzable urine solids. The results of analytic and chromatographic studies on this material are reported. About 80 per cent of the weight of urine solids was accounted for as carbohydrate components and protein. Both acidic and neutral carbohydrate moieties are present in this total material.

Dr. Maxwell Schubert, Department of Chemistry, New York University College of Medicine, generously supplied pure potassium chondroitin sulfate from cartilage, and potassium hyaluronate from umbilical cord. Dr. Harold F. Walton, Department of Chemistry, University of Colorado, Boulder, performed the analyses for ash, total sulfur, and sulfate sulfur. Major David Auld, Department of Pathology, Fitzsimons Army Hospital, assisted in the tests for blood group activity. Mr. Richard Harding performed many of the nitrogen analyses. Mr. Eugene Kanabrocki, Hines Veterans Administration Hospital, Hines, Illinois, provided many of the details upon which the hexuronic acid method was based. The Department of Medical Illustration, Subsection, Fitzsimons Army Hospital, took Ektachrome color photographs of the chromatograms and made the black and white reproductions that appear in this paper.

We are indebted to Dr. Richard J. Winzler, Department of Biochemistry, University of Illinois, College of Medicine, for critically reviewing this manuscript.

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THE PERSISTENCE IN THE BLOOD OF THE RADIOACTIVE LABEL OF ALBUMINS, GAMMA GLOBULINS, AND GLOBULINS OF INTERMEDIATE MOBILITY

III. Comparison of Die-away Plots Following Oral and Intravenous Administration of the S^{35} Label to the Same Subjects

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INTRODUCTION

THIS is the third report from an inquiry as to whether turnover rates of electrophoretically separated plasma protein components may yield insights into disease mechanisms as fruitful as have the customary measurement of concentrations.

For fractionation we standardized a sandwich type of paper electrophoretic medium from which protein components are extracted almost quantitatively for determination of specific radioactivities as well as gravimetric concentrations. In addition to albumins and gamma globulins, we have studied the alpha$_1$ alpha$_2$ beta components as a mixture.[1, 2] In certain instances, fibrinogen separated by a modification of Morrison's[3] method has been studied.

While such fractionation is crude, we have not turned to biologically more specific and chemically more complicated fractionation until certain basic problems in the interpretation of die-away radioactivity plots have been elucidated.

The initial reports from this inquiry[1, 2] dealt with these problems and found us in the following situation:

A. As calculated from the die-away plots, the apparent turnover times of albumins and gamma globulins in subjects with normal blood levels of these components proved considerably longer when autosynthesized after oral feeding of S^{35} than the apparent turnover times following intravenous injection of homologous albumins and gamma globulins labeled in vitro with I^{131} in these same subjects.

Moreover, the apparent turnover times following S^{35} ingestion were occasionally longer than the sparse measurements following oral ingestion of N^{15} reported by London and co-workers.[8]

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We are grateful to the Otho S. A. Sprague Memorial Institute for funds to initiate this series of studies; to Miss A. Gomoll and Miss G. Robey for technical assistance; and to Miss J. Peters and Mrs. R. Honig of the Neurological Service at Cook County Hospital for metabolic nursing assistance.

We are also indebted to Warden Fred Hertwig for his interest in the development of the investigational facilities on the clinical services of the Cook County Hospital.

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B. Low concentrations of a given component did not necessarily correlate with a slowing of apparent turnover times. Indeed the shortest albumin apparent turnover time was noted for the hypoalbuminemia of a subject with severe constrictive pericarditis. The longest apparent times for all components were found in the presence of nitrogen retention with no significant hypoproteinemia.

C. Among six subjects without suspected metabolic abnormality involving the plasma proteins, the range of variation of apparent turnover times proved so great as to raise doubt on possibility of clinical interpretation of comparable studies in disease states. Thus, while the apparent turnover times for the crude mixture of alpha-beta globulins proved in each subject considerably shorter than the albumins and gamma globulins times, in these subjects the range for albumins was between thirty-five and seventy days, and for gamma globulins between twenty-seven and sixty-five days. Despite good technical reproducibility, when a measurement yields 100 per cent variation in subjects in whom no clinical abnormality is suspected, there is great likelihood of unevaluated factors. The underlining of "apparent" in reporting such turnover time data is thus clearly indicated.

Of the unevaluated possibilities, two are prominent: 1. Apparent turnover times based on die-away radioactivity plots of in vitro I^{131}-labeled proteins injected intravenously may not be representative of the same protein components if they have been synthesized in the subject's own body. Sterling[4, 5] used immunologic data to rule out this possibility for I^{131}-labeled albumins. Since then several workers[6] have shown that albumin preparations with ultracentrifugal abnormalities have unusually short apparent turnover times. There still remains a faint possibility that subtle changes could be produced by an in vitro labeling procedure which would not show up in the ultracentrifuge or comparable gross physical chemical tools.

2. It is likewise possible that when an isotopically labeled amino acid is fed, the die-away plot does not simply represent the label on the protein synthesized and delivered to the plasma pool immediately following synthesis. There may be an unevaluated but continuing contribution during the die-away period from isotope degraded from labeled plasma and organ protein.

Gould and Madden[7] in their studies on fibrinogen attempted to minimize this possibility by feeding supplementary cysteine and methionine during S^{35} die-away study. Initially we have followed the same practice. In one patient the slope of the die-away curves did not change during a period of discontinuation of this unlabeled sulfur amino acid ingestion. Therefore, it still remains possible that considerable reutilization of the S^{35} label takes place despite the unlabeled dietary supplement.

To evaluate these possibilities, we have undertaken a comparison of apparent turnover times following oral ingestion of S^{35} with apparent turnover times of the same electrophoretic components in the same subjects following intravenous injection of S^{35}-labeled plasma proteins (where the labeling has been carried out in vivo in another subject and the plasma protein has been subjected to no in vitro fractionation whatsoever before the injection).

CHOICE OF SUBJECTS, DOSAGE OF S^{35}, AND PLAN OF EXPERIMENT

Except as otherwise noted, oral S^{35} was administered as labeled yeast.* Our previous maximum dosage of S^{35} (500 μc) has not as a rule yielded sufficiently high plasma protein radioactivity to permit accurate counting of separated electrophoretic components following infusion of a reasonable amount of plasma so labeled into another subject. We therefore requested and received from the AEC authorization for a sixfold increase of maximum oral dosage (3 mc.) for the study here reported.

Two subjects were chosen for this oral dosage. One (J. M.) was beleived to have no metabolic abnormality involving plasma proteins. His continued residency on the County Hospital neurological service by reason of a spastic paralysis dating from childhood, his excellent general health, and his spirit of cooperation admirably fitted this and other experimental parts of our inquiry.

The second subject (E. W.) was in chronic, quite severe uremia owing to polycystic kidneys. Clinical appraisal gave no indication of either a glomerulonephritic or pyelonephritic contribution to this uremia. His immediate prognosis was judged to be long enough to permit observations of plasma protein component radioactivities during a die-away period of at least one month.

A uremic subject was chosen because the already mentioned prolongation of apparent turnover times in our previously studied patients with uremia[1] emphasized the possibility that the well-known deficiency in excretion of sulfate and many other substances in this condition might lead to exaggerated reutilization of the isotopic label during the die-away period. (Shortly after the completion of observations on E. W., progressive diminution in urinary output was followed by death. Post-mortem examination confirmed the initial diagnostic appraisal and permitted the examination of tissues for radioactivity reported as follows.)

From each of these two subjects, two plasmaphereses approximating 200 c.c. each were carried out in the first five days following the oral isotope administration. Samples of each were taken for determination of concentrations and radioactivity levels of the electrophoretic components under study. The remainder of each plasmapheresis was injected intravenously without any in vitro fractionation as follows:

1. From each donor, 1 plasma was injected intravenously into a subject (a) whose chronic neurological condition was not considered related to any abnormality involving plasma proteins; (b) without evidence of renal disease; and (c) in whom apparent turnover times of the three electrophoretic components had been measured previously following oral administration of S^{35}. In addition, apparent turnover times of gamma globulins had been measured following intravenous injection of the I^{131}-labeled fraction.* Of these two, A. W.'s findings have been already reported; V. B. suffered from long-standing chorea of the Huntington type.

2. Into a uremic subject chosen for chronicity and prognosis on the same basis as was the uremic donor. On these subjects previous isotopic studies had not been carried out. Of the two uremic recipients, A. A., on clinical appraisal, was classified as malignant nephrosclerosis. S. G. was classified as a chronic glomerulonephritic. In both, the possible contribution of superimposed chronic pyelonephritis was difficult to evaluate. S. G. gave no evidence of amyloidosis nor of multiple myeloma; A. A. gave no evidence of lupus erythematosis by usual laboratory criteria. Both were discharged as ambulatory at the end of the study.

The donor and both recipient uremics exhibited nonprotein nitrogen levels greater than 100 mg. per cent, creatinine levels greater than 5 mg. per cent, and plasma protein partitions by the Howe method within normal limits during this period of study.

The plan of infusion then may be summarized thus:

Donors:	J. M. (''normal'')		E. W. (uremic)	
Recipients:	G. B. (''normal'')	A. A. (uremic)	A. W. (''normal'')	S. G. (uremic)

*Obtained from Abbott Laboratories, North Chicago, Ill.

Serial sera were withdrawn for a period of about one month on the two donors and the four recipients. Methods of fractionation, extraction, measurement of component concentration, and radioactivity were carried out as described in our previous publications[1, 2] and die-away plots were made of the resultant data in the customary semilogarithmic fashion against time.

RESULTS

A. *Albumins.*—Fig. 1 presents the die-away plots of Donor J. M. (normal) following S^{35} orally; Recipient G. B. (normal) following S^{35} orally; Recipient A. A. (uremic) following infusion of J. M.'s plasma; Recipient G. B. (normal) following infusion of J. M.'s plasma. The apparent turnover times ($t\frac{1}{2}/1n2$ as estimated from the slopes between the fourth and twentieth days) are indicated for each line.

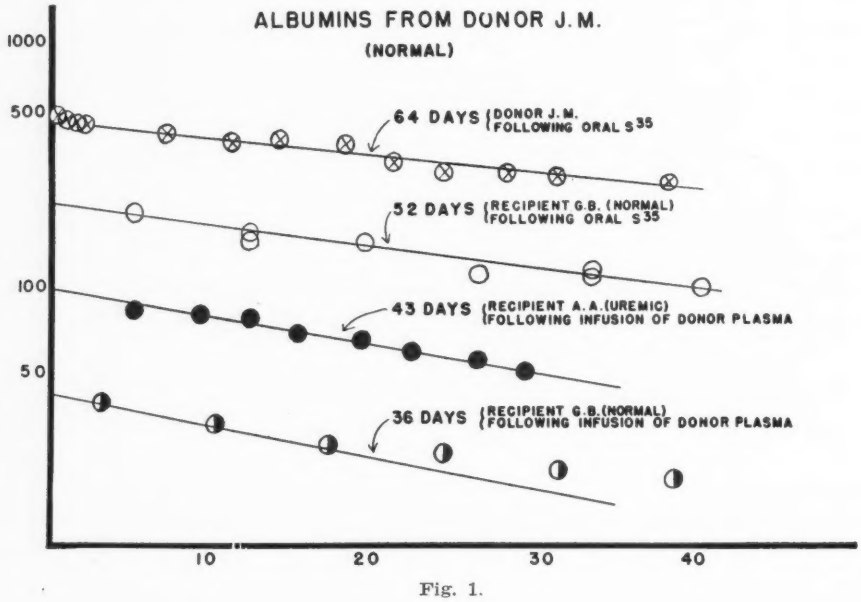

Fig. 1.

The scatter of the experimental points about the lines gives visual indication of the expected accuracy of the apparent turnover times. Thus we do not consider the difference between the values for the first two slopes (64 and 52 days) to be beyond the combined experimental errors of the methods; we do consider the differences between these values and that for the bottom initial slope (36 days) are well beyond the experimental error range as determined in other experiments by measurements in triplicate.

The eye gives clear evidence of the progressive steepness of the slopes of these plots as one goes from the top to the bottom of Fig. 1.

Also the eye gives indication of some flattening of the bottom slope in the last part of the plotted period, such that it becomes comparable to the top two initial slopes. Neither of these, however, shows evidence of final flattening.

Fig. 2 presents the albumins' die-away plots relevant to Donor E. W.'s (uremia) albumins. While, of the four plots, only the bottom shows evidence of final flattening, the trend of increasing steepness of slope from the top to the bottom of the figure is accentuated in comparison with Fig. 1, by reason of the essential flatness of the top plot (donor's albumins following oral S^{35}). The second plot (S. G., the uremic recipient) has a slope differing little from that of A. A., the uremic recipient of Fig. 1, but very significantly steeper than that for the donor's. The albumins' plot for the "normal" recipient (bottom) is not only steeper than the plot for this same subject after oral S^{35} but approximates in its apparent turnover time values that have been reported following intravenous injection of I^{131}-labeled albumin.

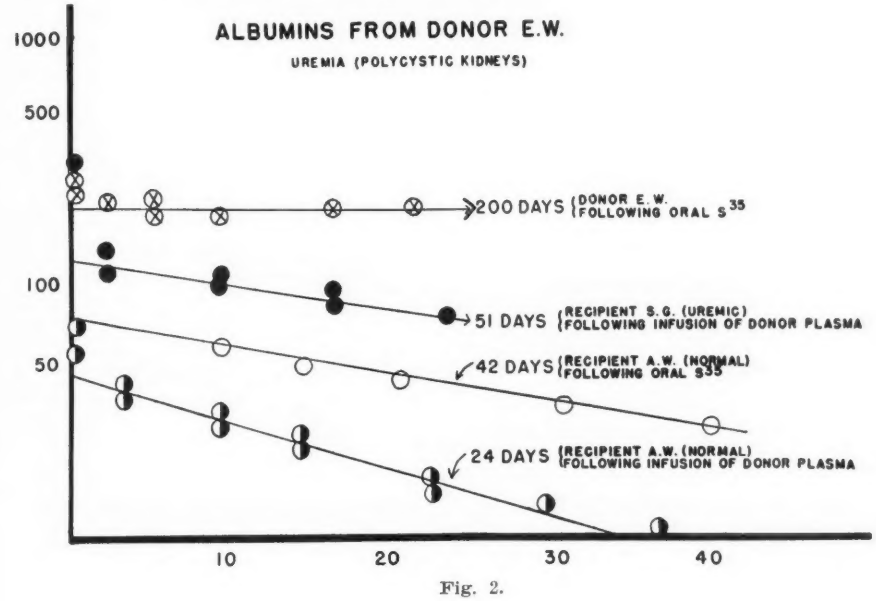

Fig. 2.

The results for albumins may be visualized thus:

Donors:	J. M. ("normal") 64 days		E. W. (uremic) 200 days	
Recipients:	G. B. ("normal") 36 days	A. A. (uremic) 43 days	A. W. ("normal") 24 days	S. G. (uremic) 51 days

In summing up, (a) the apparent turnover times of the albumins for both recipients were less than the corresponding donors; (b) in each experiment the apparent turnover time for the uremic recipient following infusion of the donor plasma was longer than that for the paired "normal"; and (c) the apparent turnover time of each normal recipient was significantly shorter than when estimated in the same subject following S^{35} ingestion.

B. *Gamma Globulins.*—Fig. 3 presents the die-away plots relevant to the gamma globulins from "normal" donor J. M. Here the slopes for Recipients

A. A. (uremic, next to bottom) and G. B. (''normal,'' bottom) are clearly steeper than that for the donor following oral S^{35} (top). Moreover, both lower plots exhibit flattening of slope during the latter part of the experimental period.

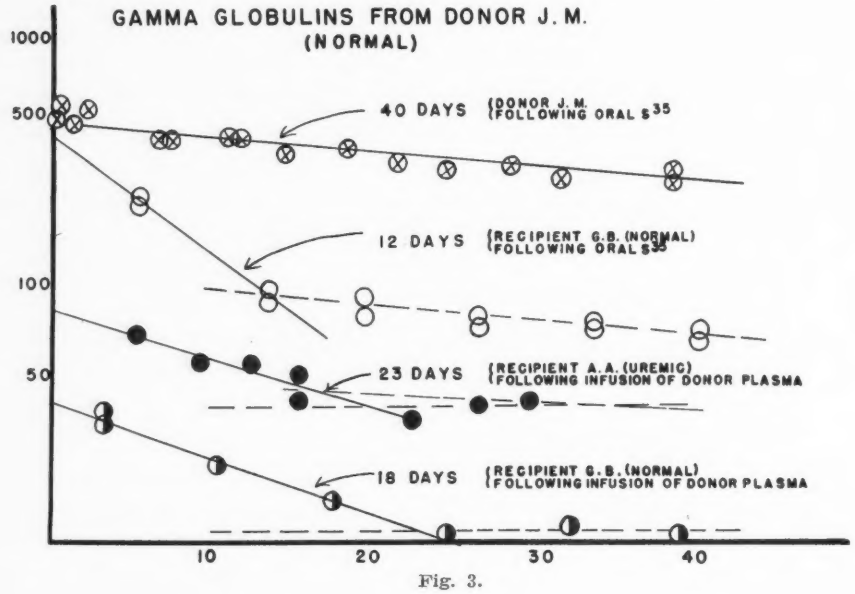

Fig. 3.

The most striking flattening is seen in the plot for the ''normal'' recipient (G. B.) following oral S^{35}. Here the initial steepness of slope yields an apparent turnover time (12 days) shorter than that (18 days) estimated by injection of I^{131}-labeled gamma globulins in the same subject.

This is the only instance in our experience of an apparent turnover time for any fraction following oral S^{35} as short or shorter than the time measured following intravenous injection of the fraction labeled in vitro with I^{131}. Due to an unfortunate laboratory accident, this slope is based on only two blood samples on the fifth and fourteenth days, respectively.

In Fig. 4 are presented the die-away plots relevant to the gamma globulins from Donor E. W. As was the case with the albumins, the gamma globulins of this uremic subject following oral S^{35} (top) show a flatter slope than those of ''normal'' Donor J. M. under the same circumstances (Fig. 3, top). Both the uremic (S. G.) and the ''normal'' (A. W.) recipients exhibit steeper slopes during the first part of the experimental period, and both show final flattening. Likewise, the slope for A. W. is steeper than that of his own gamma globulins measured following oral S^{35} (third from top).

The results for gamma globulins may be visualized thus:

Donors:	J. M. (''normal'') 40 days		E. W. (uremic) 95 days	
Recipients:	G. B. (''normal'') 18 days	A. A. (uremic) 23 days	A. W. ("normal") 23 days	S. G. (uremic) 23 days

These relationships between the apparent turnover times follow much the same pattern noted previously for the albumins.

The figures of the last line are all very close to the values determined in G. B. and A. W. by I^{131} intravenous gamma globulins (18 and 23 days, respectively).

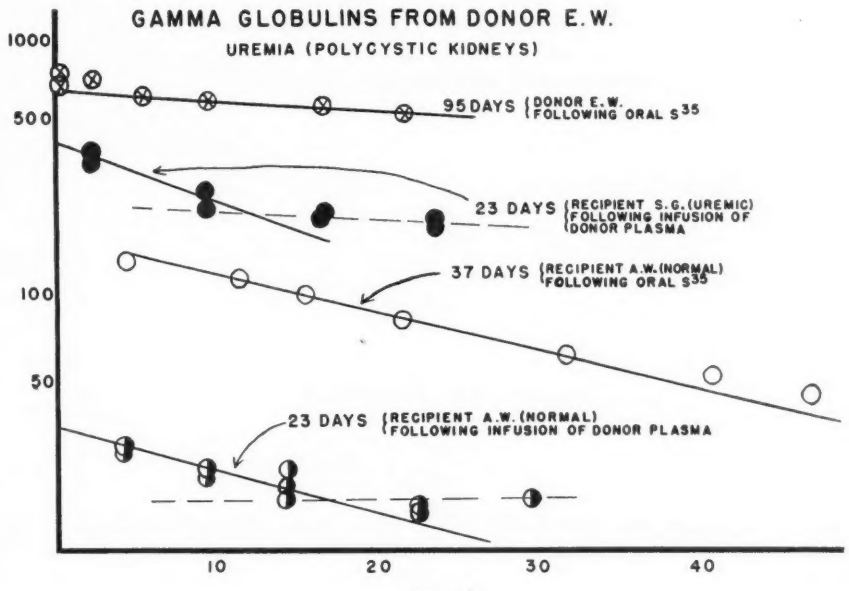

Fig. 4.

C. *Globulins of Intermediate Mobility.*—Fig. 5 presents the die-away plots relevant to this fraction from Donor J. M. (''normal''); Fig. 6, comparable data for Donor E. W. (uremia). In both of these figures the initial top slopes are much steeper than those noted for either albumins or gamma globulins in the same subjects. This is in keeping with the results already reported on this fraction. The slope for the ''normal'' donor following S^{35} ingestion is only slightly steeper than that of the uremic donor. Six out of the eight slopes in these two figures show striking final flattening, as indicated by dotted lines.

The results for globulins of intermediate mobility may be visualized thus:

Donors:	J. M. (''normal'') 12 days		E. W. (uremic) 16 days	
Recipients:	G. B. (''normal'') 8 days	A. A. (uremic) 25 days	A. W. (''normal'') 6 days	S. G. (uremic) 12 days

The results of these paired experiments are different from those for albumins and gamma globulins in the following respects.

(a) Both normal recipients showed essentially identical apparent turnover times which are significantly shorter than those of the corresponding donors

following oral S^{35} or of their own autosynthesized $alpha_1$ $alpha_2$ beta fraction following oral S^{35}; (b) in Fig. 5 the uremic recipient showed slightly longer apparent turnover time than that of corresponding "normal" donor following oral S^{35}. In Fig. 6 the uremic recipient's apparent turnover time is only slightly shorter than that of the corresponding uremic donor under the same circumstances.

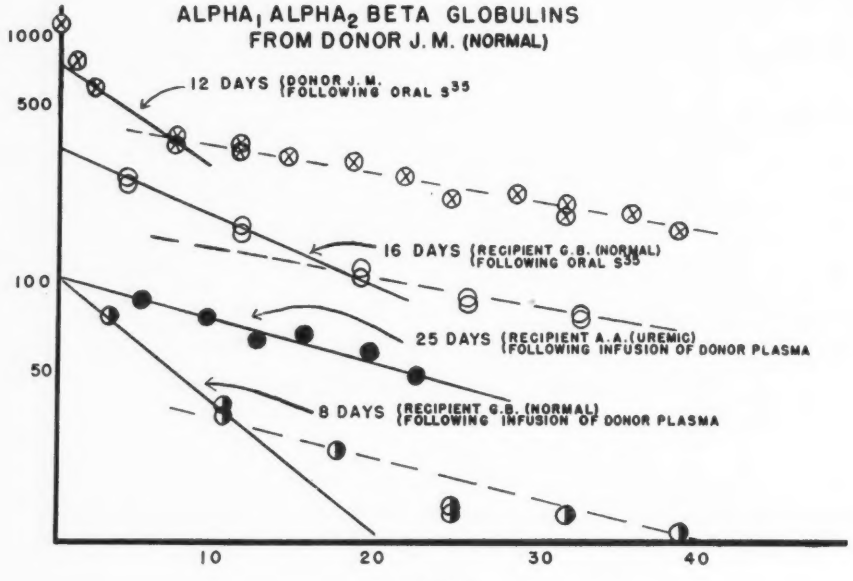

Fig. 5.

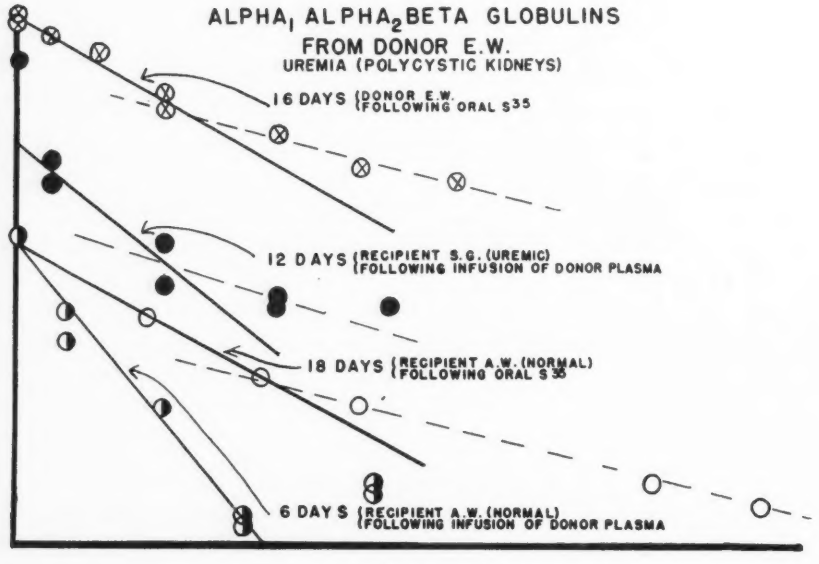
ALPHA1 ALPHA2 BETA GLOBULINS
FROM DONOR E.W.
UREMIA (POLYCYSTIC KIDNEYS)
16 DAYS (DONOR E.W. (FOLLOWING ORAL S35
12 DAYS (RECIPIENT S.G. (UREMIC) (FOLLOWING INFUSION OF DONOR PLASMA
18 DAYS (RECIPIENT A.W. (NORMAL) (FOLLOWING ORAL S35
6 DAYS (RECIPIENT A.W. (NORMAL) (FOLLOWING INFUSION OF DONOR PLASMA

Fig. 6.

D. *Fibrinogen.*—The die-away plots relevant to fibrinogen from Donor J. M. are presented in Fig. 7. Here, all four plots show final flattening as indicated by the dotted lines. The initial slopes both of the donor (top line) and of both recipients are identical and correspond to an apparent turnover time of eight days. This value is considerably shorter than the seventeen days estimated from the die-away plot of the normal recipient after oral S^{35}.

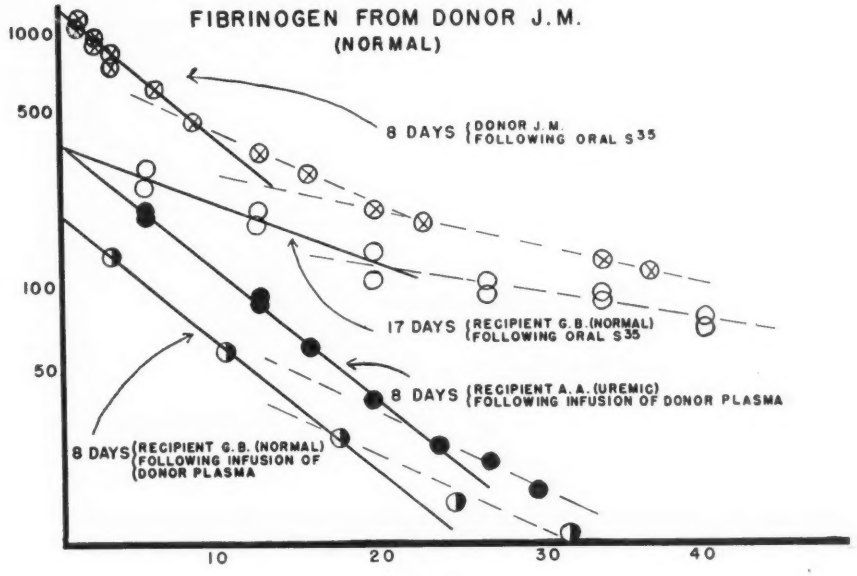

Fig. 7.

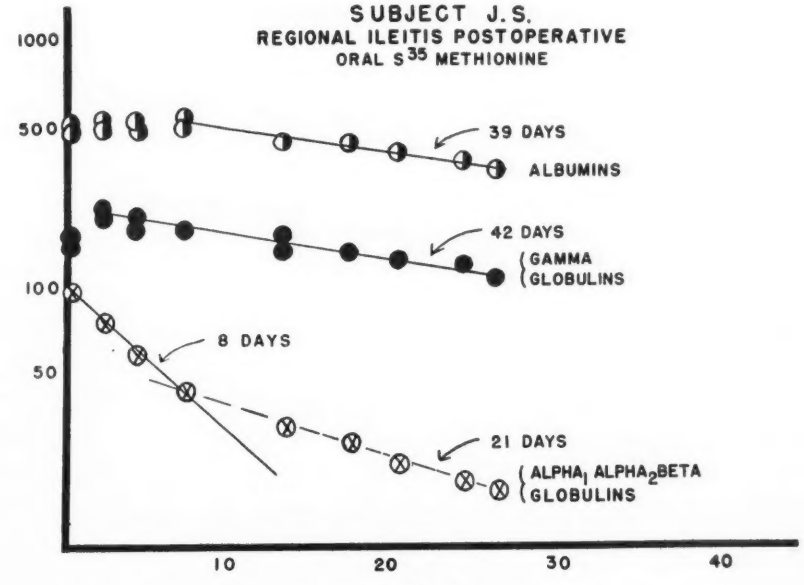

Fig. 8.

E. *Estimates of Apparent Exchangeable Pools.*—The numerical scale given in Figs. 1 to 8 for the ordinate (counts per minute) is an arbitrary one for convenience in spacing of the plots. If the arbitrary scale is replaced by the specific radioactivity corrected for radioactive decay to the day of plasma infusion, and if the amount of each injected electrophoretic component and its specific radioactivity is known, the intercepts of the linear portion of the die-away plots on the ordinate may be used to estimate the total exchangeable pools of these components. For, if the extrapolated slope of the die-away plot is truly related to metabolic turnover time, this intercept gives the specific radioactivity of the component in question as though equilibrated distribution were complete at the time of infusion, and thus the magnitude of the pool may be estimated by the observed dilution of the injected component.

TABLE I. TOTAL APPARENT EXCHANGEABLE POOLS (GRAMS) ESTIMATED FOLLOWING INTRAVENOUS S^{35}-LABELED PLASMA*

	ALBUMIN		$ALPHA_1$ $ALPHA_2$ BETA MIXTURE		GAMMA GLOBULINS		FIBRINOGEN	
SUBJECT	EXTRA-VASC.	INTRA-VASC.	EXTRA-VASC.	INTRA-VASC.	EXTRA-VASC.	INTRA-VASC.	EXTRA-VASC.	INTRA-VASC.
A. W.	160	80	80	50	80	50		
S. G.	220	80	150	60	120	40		
G. B.	260	90	60	60	40	30	8	8
A. A.	250	60	450	50	140	40	3	10

*The intravascular estimate is made by multiplying the measured plasma concentration by an assumed plasma volume of 2,500 c.c.

The extravascular estimate is made by subtraction of the intravascular figure from the intercept on the ordinate of the solid lines in Figs. 1 to 8 as described in the text.

All figures have been rounded to the nearest 10 Gm.

Now the estimates thus made and given in Table I are not presented under any quantitative assumption relating slopes of the die-away plots used for extrapolation to true turnover times. Not only do we have no basis for such an assumption, but depending upon the treatment of the final flattened part of some of these plots (for which see the following), estimates would prove significantly different. We present these estimates merely to indicate that the order of magnitude for albumins is comparable with previous reports following injection of the I^{131}-labeled fraction[4, 5]; that extravascular exchangeable pools of significant magitude for both gamma globulins and certain globulins of intermediate mobility appear to exist. With reference to fibrinogen, in that an extravascular pool has not ordinarily been suspected, it should be pointed out that any unobserved clots caught in the filter of the plasma administration set would result in a falsely high estimate.

DISCUSSION

Die-away plots of the type illustrated in Figs. 1 to 8 are usually represented mathematically by a sum of exponentials. Any term in this sum gives a linear semilogarithmic plot whose negative slope is proportional to the "apparent turnover time" of the processes represented. The significance of the intercept on the ordinate at zero time (i.e., time of isotope administration) depends upon what process the term is believed to represent.

For this type of study, ordinarily one thinks of a die-away plot as divisible into segments representing these processes: (1) The immediate mixing within the blood stream. (2) The equilibration of the injected component with the extravascular exchangeable pool. Usually complete within three days, the intercept of the early part of the segment of the plot on the ordinate provides the familiar measure of plasma volume. Neither of these processes is germane to this study. (3) The metabolic degradation of the isotopically labeled protein component.

Now, (a) if rate of synthesis is equal to that of degradation,* and (b) if there is no reutilization of degraded isotope in resynthesis of the component during the experimental period, and (c) if the multitude of the molecular species in the electrophoretic component are characterized by closely similar turnover rates, this segment of the plot should be linear. Deviation from linearity thus can result from deviations from any of these specifications.

A glance at Figs. 1 to 8 emphasizes that it is one thing to describe segments of an idealized die-away plot but it is entirely another thing to take experimental data on as heterogenous group of molecular species as an electrophoretic component and divide the actual plot into such segments with a great degree of sureness.

We believe that the discrepancies between apparent turnover times and the final flattening of die-away plots described previously are best attributable to a considerably greater reutilization of S^{35} for resynthesis of protein during the die-away period when S^{35} is given orally than when S^{35}-labeled proteins are infused intravenously. This view is consistent with the recent finding of Humphrey and McFarlane[10] of shorter apparent turnover times following intravenous injection of in vitro fractionated C^{14}-labeled normal and immune globulins into the rabbit than the values previously reported by Schoenheimer[11] and Heidelberger[12] following oral N^{15}.

Of course with respect to animals as well as to human beings, one cannot completely dismiss the possibility that an individual turns over plasma proteins from another individual of the same species faster than he does those synthesized in his own body.

We are forced to minimize this possible basis for our results for these reasons: Recently we have had the opportunity to determine radioactivity levels of tissues recovered at autopsy from E. W. of this study and from another nonuremic subject whose plasma proteins had been labeled with oral S^{35} methionine only eight days before death. As measured on samples washed free of blood, although not perfused, dried from the frozen state, and counted in a

*Steinbock and Tarver[9] have presented in useful tabular form the interrelations of directions of change for synthesis and degradation rates, replacement rate, exchangeable pool size, and apparent turnover time when the subject of study is presumably not in the steady state.

In that the normal subjects of our own study were in as steady a state as is achievable under conditions short of a complete balance study, these considerations are not applicable.

While the uremic subjects were likewise chosen for stability of their clinical status, it is quite possible that despite maintenance of unchanging concentrations of the components under study during the experimental period, the observed prolongation of apparent albumins' turnover time in the presence of an apparently subnormal total exchangeable pool reflects a long-standing depression of rate of synthesis in the presence of normal degradation. Serial measurements of a diminishing exchangeable pool would be necessary to establish this interpretation.

fashion analogous to that for plasma protein fractions, radioactivities were not more than 50 per cent below, and in many instances were comparable to the specific activities of plasma protein fractions obtained the day before death. Radioactivity of this order (shared alike by those extrahepatic tissues believed involved in gamma globulin synthesis[13, 14] as well as by the liver) is evidence for the fairly rapid development of a very substantial amount of isotopically labeled tissue protein whose own metabolic turnover could readily release isotope for plasma protein resynthesis.

Valid estimation of a metabolic turnover time thus requires the slope of that segment of the die-away plot where such resynthesis is minimal. The flattening noted after two or three weeks in many of the illustrated plots suggests an optimal period between about the fifth to the fourteenth day. If the dotted lines (representing the latter weeks) of the bottom plots of Figs. 3 and 4 are extended backward through this period, and for each day the specific radioactivity values are subtracted from the values indicated by the solid lines, a semilogarithmic plot of the differences against time yields a slope "corrected" for a contribution of resynthesis of assumed identical magnitude between days five and fourteen and later in the die-away period.

Apparent turnover times for the intravenously injected S^{35}-labeled gamma globulins of G. B. and A. W. emerge from this "correction" as shorter than times estimated from intravenous I^{131}-labeled gamma globulins in the same subjects! Before correction they were identical.

Applied to the bottom two plots of Fig. 7, this "correction" leads to apparent turnover times more of the very short order suggested by the older acute hepatectomy experiments rather than the order of eight days suggested by the die-away plots of Madden and Gould[7] following S^{35} feeding. Possibly because their plots do not extend beyond three weeks, these authors did not observe the terminal flattening seen in our longer studies, and thus the "correction" cannot be applied to their data.

To the general application of this "correction" for die-away plots following oral S^{35}, our own data present these further obstacles: (a) some plots continued for as long as six weeks show no terminal flattening (see the top plots in Figs. 1 and 2); (b) in certain studies of albumins and gamma globulins we have encountered a positive (upward) slope during the first week indicative of sufficient continuing synthesis to reverse completely this period's usually steep negative slope (mixing with the total exchangeable pool).

Illustrated in Fig. 8 (top and middle), the latter type of die-away plots rarely show the terminal flattening illustrated in the bottom plot of Fig. 8, and in our experience most frequently follow use of oral S^{35} methionine alone rather than the yeast which contains both cysteine and methionine. Volwiler,[15] many of whose recent results parallel the findings of this paper, has emphasized the differing metabolic pathways of methionine and cysteine sulphur.

Die-away plots analogous to those of Fig. 8, likewise following oral S^{35}, have been given metabolic interpretations in contrasting normal and severely burned patients by Blocker and co-workers.[16]

Because of the considerable range of variation of apparent turnover times following oral S^{35} in "normal" subjects, because intravenous in vivo labeled S^{35} fractions show considerably shorter turnover times in two of these same "normals," and because these latter values approach or equal in shortness the values previously reported by others and ourselves with in vitro I^{131}-labeled fractions, we do not believe that die-away plots following the oral isotope are the best way to achieve interpretable metabolic turnover data at this time. If in vivo synthesis is to be used, intravenous administration of the protein avoids to some extent the pitfalls of a large labeled tissue protein pool.

It remains possible that the quantitative analysis of the period of synthesis in the hours following oral administration of the isotope will prove more fruitful. This we have under exploration at present.

SUMMARY

In further investigation of the discrepancies in apparent turnover times when estimated by in vitro-labeled I^{131}-labeled protein fractions injected intravenously and when estimated following autosynthesis after oral S^{35}-labeled yeast, two subjects were given enough oral yeast so labeled to permit radioactivity "die-away" plots of their autosynthesized plasma proteins not only in themselves but following intravenous infusion of their plasmas into two other subjects. Plasma proteins so infused were (in contrast to I^{131} labeling) "in vivo" labeled and were subjected to no in vitro fractionation prior to infusion.

Of the two donors, one was without known nutritional or metabolic abnormality; the other was a chronic uremic, chosen because of unusually prolonged apparent turnover times following S^{35} ingestion previously noted in this state. From each donor plasma was infused into a comparable pair of recipients, a "normal" and a chronic uremic.

With minor exceptions, the results for albumins, gamma globulins, fibrinogen, and a crude $alpha_1$ $alpha_2$ beta mixture exhibited the following pattern: (a) apparent turnover times in the recipients following infusion shorter than both those in corresponding donors and (when available) than those estimated in the same subjects following oral isotope administration; (b) apparent turnover times for both uremic donors and recipients longer than for corresponding "normals"; (c) flattening of slope of certain donor and recipient die-away plots during the latter part of the study.

The role of isotope reutilization in yielding these results is appraised in connection with autopsy tissue radioactivity findings in two subjects who had received oral S^{35}. Note is made of the identity of apparent turnover times following in vitro-labeled I^{131} and in vivo-labeled S^{35} gamma globulin in two subjects in reaching the conclusion that the intravenous route is preferable to the oral route in achieving interpretable turnover data on plasma proteins.

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LEUKOCYTE RESPONSE TO LEUKOCYTE NUCLEAR AND CYTOPLASMIC ANTISERA

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LEUKOCYTES possess antigenic properties which make possible the production of antisera of considerable cell type specificity, but little is known concerning the cytologic components of leukocytes which are responsible for their antigenic properties. The studies to be reported here were undertaken to determine the capacity of leukocyte nuclei and cytoplasms to provoke antibody responses, and to evaluate the hematological effects in vivo of such antinuclear and anticytoplasmic sera.

EXPERIMENTAL PROCEDURE

Preparation of Leukocyte Fractions.—Twelve guinea pigs weighing from 300 to 350 grams were injected intraperitoneally with 15 ml. of sterile 4.0 per cent beef broth. Twelve hours later exsanguination was performed by cardiac puncture, the peritoneal cavity was opened, the exudates were withdrawn aseptically into heparinized syringes, and they were pooled in a sterile tube. The spleen from each animal then was removed and stored in a separate sterile container.

The total volume of the pooled exudates was 50.0 ml. and contained about 70,000 cells per cubic millimeter. Smears of these leukocytes showed 85 to 90 per cent polymorphonuclear leukocytes, 10 to 15 per cent mononuclear cells, and a few erythrocytes. Following centrifugation of the pooled exudates at 600 g. for 10 minutes the supernatant was removed and to the 6.0 ml. of packed leukocytes in the sediment 9 volumes of sterile 0.18 M sucrose solution was added and mixed. This material was homogenized for ten minutes in a glass Potter homogenizer, and then centrifuged at 600 g. for ten minutes. The supernatant was removed, the sediment was resuspended in sucrose, homogenized again, and respun at 600 g. The supernatants were combined and smears revealed that they contained only granular cytoplasmic debris and no cell nuclei or whole cells. The sediment consisted mostly of intact nuclei of polymorphonuclear leukocytes to which a lacy network of cytoplasmic material, depleted of its contents, remained attached. Rarely intact polymorphonuclear leukocytes, mononuclear cells, and erythrocytes were observed in the nuclear fraction.

The spleens removed from a total of 16 guinea pigs were passed through a small garlic press containing openings 1.0 mm. in diameter. The weight of this splenic pulp was 12.0 grams. The material then was transferred to a Potter homogenizer with 9 volumes of 0.88 M sucrose solution, homogenized for twenty to thirty minutes, spun at 600 g. for ten minutes,

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and the supernatant removed. The sediment was resuspended in sucrose solution, homogenization and centrifugation were repeated as before, and the supernatants were combined. Smears of these fractions again indicated the presence only of granular cytoplasmic debris in the supernatant, and very little except the intact nuclei of lymphocytes along with a few erythrocytes and reticular cells in the sediment. These nuclei appeared to be completely denuded of cytoplasm.

The supernatants obtained from the centrifugation of the fractionated granulocytes and splenic cells represented the cytoplasmic fractions and the sediments represented the nuclear fractions. The volume of each cytoplasmic fraction was about 60 ml., and each nuclear fraction was made up to a volume of about 60 ml. with sterile physiologic saline.

Preparation of Antisera.—During the following three weeks aliquots of the cellular fractions were given by intravenous injection to albino rabbits at biweekly intervals. Ten milliliters of the granulocyte cytoplasmic fraction was given at each injection to one rabbit, and a similar amount of the spleen cytoplasmic fraction was given to a second rabbit. Five milliliter portions of splenic nuclear fraction were administered at each injection to two rabbits, while two other rabbits received similar amounts of granulocytic nuclear fraction. During the period of immunization one of the rabbits receiving the granulocytic nuclear fraction died. Ten days after the last injection aseptic exsanguination was carried out on each remaining rabbit, and the sera were separated. The four types of serum obtained henceforth were designated as "granulocytic nuclear antiserum," "granulocytic cytoplasmic antiserum," "splenic cell nuclear antiserum," and "splenic cell cytoplasmic antiserum."

Administration of Antisera.—On the day on which these antisera were obtained, either 2 ml. or 3 ml. portions of each was administered intraperitoneally to 5 normal guinea pigs. During the following ten days frequent total and differential leukocyte counts and hemoglobin determinations were performed on capillary blood. Hemoglobin determinations were performed colorimetrically by the method of Evelyn[1] and leukocyte counts were made in duplicate.

RESULTS

General Response.—Almost immediately following the administration of the heteroimmune rabbit serum, most of the guinea pigs developed a profound shock-like state which lasted from twelve to twenty-four hours. During this period of time the animals appeared lethargic, capillary blood was dark in color, and flow was scanty. A few animals died during this initial shock phase; however, those which survived usually remained well during the remaining period of observations. The severity of the initial shock did not bear relationship to the type of antiserum employed.

Granulocytic Nuclear Antiserum.—Following the injection of granulocytic nuclear antiserum to five guinea pigs there developed rapidly a fall in the number of circulating lymphocytes, the maximum drop occurring seven hours after the injection. Recovery was complete and sustained within twenty-four to forty-eight hours. (Fig. 1.) In 3 of the 5 animals there occurred a mild granulocytopenia lasting from three to seven days followed by a polymorphonuclear leukocytosis in most instances (Fig. 2). Except for the appearance of a few metamyelocytes there were no significant alterations in leukocyte morphology.

Granulocytic Cytoplasmic Antisera.—Subsequent to the administration of granulocytic cytoplasmic antisera to 5 guinea pigs, there again occurred a prompt lymphopenia, maximum during the first twenty-four to forty-eight hours, followed by recovery to normal levels (Fig. 1). In contrast with the granulocytic

nuclear antiserum, this antiserum rapidly produced a severe granulocytopenia in all animals which was well maintained in 2 for a period of ten days, and in the other 3 for five days (Fig. 2). The animals with granulocyte depression for ten days received 3.0 ml. of antiserum while those lasting five days had received 2.0 ml. of antiserum. A few metamyelocytes appeared in the peripheral blood of 2 animals between the fifth and seventh post injection days. A mild anemia, maximum on the fifth day, was observed in all animals, recovery being complete by the tenth day.

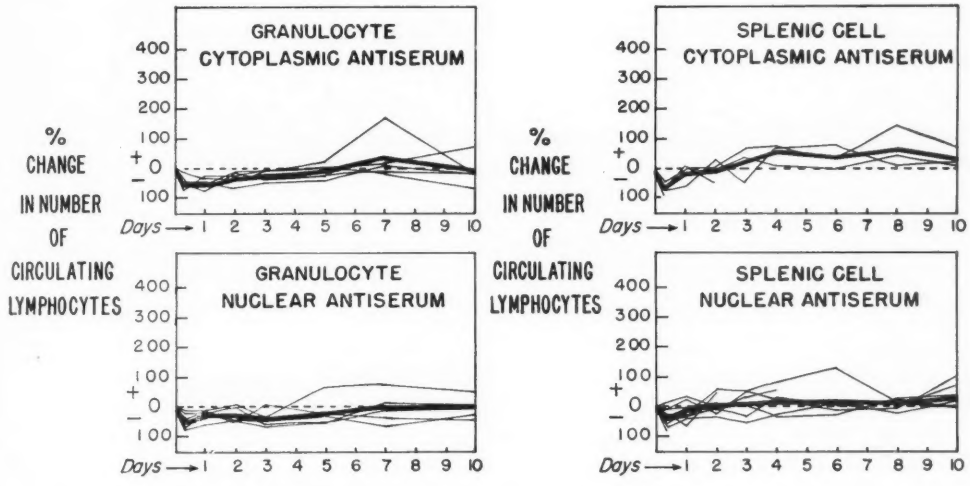

Fig. 1.—The heavy line in each graph represents the average percentage change in numbers of circulating lymphocytes in normal guinea pigs following the administration of rabbit leukocyte antisera. The thin lines represent individual guinea pig responses.

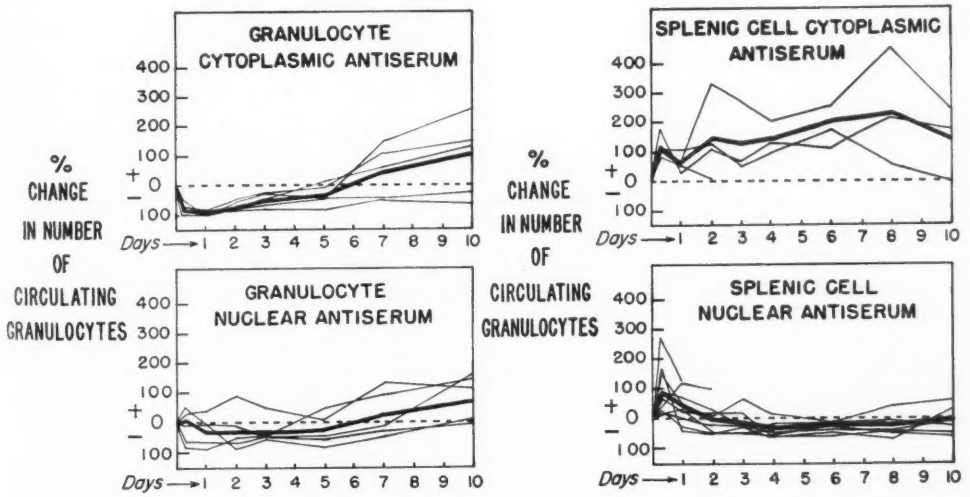

Fig. 2.—The heavy line in each graph represents the average percentage change in number of circulating granulocytes in normal guinea pigs following the administration of rabbit leukocyte antisera. The thin lines represent the responses of individual animals.

Splenic-cell Nuclear Antiserum.—A total of 10 guinea pigs was injected with splenic-cell nuclear antiserum, 5 with the antiserum obtained from 1 rabbit and 5 with antiserum from the other. Three of these guinea pigs died within the first forty-eight hours. The postinjection hematological changes observed in the two groups were essentially the same, and the results are to be considered together. The initial lymphopenia which occurred in these guinea pigs was less profound than with either of the granulocytic antisera (Fig. 1). Recovery was equally as prompt, and in no instance was there maintenance of significant depression of the number of circulating lymphocytes. A moderate to marked increase in the number of circulating granulocytes within the first twenty-four hours was observed in 9 to 10 animals followed by a return to normal or slightly subnormal levels in most animals during the ensuing nine days (Fig. 2). In 7 of the 10 animals variable numbers of prolymphocytes, lymphoblasts, or lymphocytes undergoing mitosis were found in the peripheral blood following the injection of antiserum. These cells appeared as early as twenty-four hours postinjection and were found in some animals up to the tenth day. They usually constituted from 2 to 5 per cent of the differential count, but levels as high as 14 per cent were seen. A moderate increase in the number of circulating Foa-Kurlov cells was noted in a few animals. A moderate anemia, maximal on the third postinjection day, was present in about one-third of the animals.

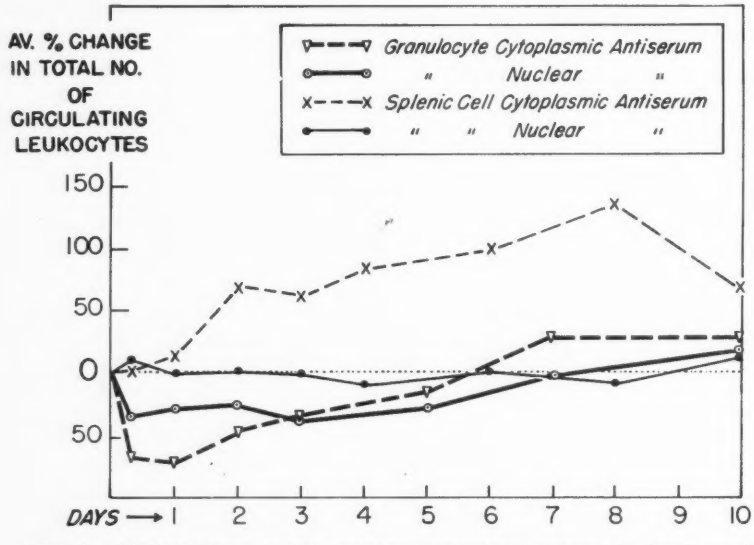

Fig. 3.—Plotted above is the average percentage change in total number of circulating leukocytes in each group of guinea pigs following the administration of rabbit leukocyte antiserum.

Splenic-cell Cytoplasmic Antiserum.—Only 3 of the 5 guinea pigs injected with splenic-cell cytoplasm antiserum survived for more than forty-eight hours. All animals developed a moderate to severe lymphopenia within the first twenty-four hours, but in none was it maintained for a longer period of time (Fig. 1). In 2 of the 3 animals a moderate lymphocytosis lasting from eight to ten days

occurred. The most striking finding was the prompt development and maintenance of increased numbers of circulating granulocytes in all animals during the period of observation without significant alterations in leukocyte morphology (Fig. 2). Two of the 3 animals surviving for ten days developed a moderate anemia which disappeared completely by the end of the study period.

Other Hematological Observations.—No significant or consistent change was observed in the number of circulating monocytes, basophils, or eosinophils in the guinea pigs following the administration of these antisera. The anemias which occurred were normocytic and normochromic in type. Nucleated red cells (metarubricytes) were found in small numbers on the peripheral blood smears made between the third and eighth postinjection days of the more anemic guinea pigs. The occurrence of anemia in these animals was variable, and did not appear to depend upon either the type of serum administered or the severity of the initial shock phase. The changes which occurred in the total leukocyte counts following the administration of each antiserum primarily were a reflection of changes which occurred in the absolute number of circulating granulocytes (Fig. 3). The only exception to this was the maintenance of the pretreatment level of total leukocytes following the administration of splenic-cell nuclear antiserum. This was due to the absolute lymphocytosis which appeared and remained after the third postinjection day.

DISCUSSION

The hematological responses to normal rabbit sera administered intraperitoneally to guinea pigs have been reported previously.[2-4] These changes consist essentially of an increase in the number of circulating granulocytes during the first twenty-four hours to 2 or 3 times the initial level with a return to normal by forty-eight hours. A concomitant moderate to severe lymphocytopenia during the first twenty-four to forty-eight hours has been observed. It is to be emphasized that following the intraperitoneal administration of normal rabbit serum to guinea pigs the hematological changes after the first forty-eight hours rarely are significant.

Of the four different types of antisera prepared against guinea pig leukocyte fractions the most significant effect was that produced by the granulocytic cytoplasmic antiserum. The severe granulocytopenia observed in the animals receiving this antiserum was consistent in all animals, was well sustained, and could be correlated with the amount of material administered. The minimal granulocytic response to the granulocytic nuclear antiserum could be explained in several different ways: (1) that the nucleoprotein of these cells is a relatively poor antigenic stimulant; (2) that the rabbit injected was a poor antibody producer; (3) that a leukocyte antibody was produced, but was not detected by these methods; or (4) that a relatively nonspecific nuclear antibody was produced and that its leukocytic activity was neutralized by the nuclei of other organ cells. Although the total aliquot volume of cytoplasmic material injected was twice that of the nuclear fraction, the dilution of these fractions was such that the total amount of nuclear material administered to each rabbit

was several times greater than that of the cytoplasmic material. This suggests that the minimal response obtained with the nuclear antisera probably was not due to the administration of an insufficient amount of antigen. The production of potent antisera to other tissue-cell cytoplasmic fractions has been noted by others.[5, 6] Studies in mouse leukemia also indicate that splenic cytoplasmic particulate fractions, especially the mitochondria, are capable of producing antisera which is quantitatively much more active than that produced from equivalent amounts of nuclear material.[7, 8]

The responses to the administration of antisera prepared against lymphocytic cellular fractions are more difficult to interpret. The initial polymorphonuclear leukocytosis does not differ appreciably from that observed following the administration of normal rabbit serum to guinea pigs.[2-4] However, the maintenance of a polymorphonuclear leukocytosis over a period of ten days following the administration of splenic-cell cytoplasmic serum rarely occurs with normal rabbit serum, the leukocytosis almost invariably having disappeared after twenty-four hours.[2-4] It is possible that this serum contained antibody or other components which either inhibited the factors responsible for destruction and sequestration of granulocytes or stimulated the production and release of these cells from the bone marrow. The granulocyte response to the splenic-cell nuclear antisera appeared to differ little from that of normal rabbit serum. The number of animals in this group which developed an absolute lymphocytosis following the administration of splenic-cell nuclear serum is too few to justify any speculation as to mechanisms involved.

The initial lymphopenia observed in the guinea pigs following the administration of granulocytic antisera has been noted by others,[2, 9-13] and in our studies did not differ appreciably from that produced by the lymphocytic antisera. These lymphopenic responses invariably were associated with a severe shocklike state and probably represented the nonspecific stress response which develops following the administration of foreign protein.[14] The subsequent observations did not indicate a significant variation in the number of circulating lymphocytes present, although the appearance of youthful forms was of considerable interest. The failure of the splenic-cell antisera to produce a more sustained lymphopenia remains unexplained. The same possibilities exist as previously enumerated for failure of the granulocyte nuclear fraction to produce a physiologically active antiserum. However, other investigators using similar techniques have successfully produced antisera from intact splenic cells which affected lymphocytic cells both in vivo[10, 15-21] and in vitro.[7, 20-25]

The transient mild anemias observed in the guinea pigs following the administration of heteroimmune leukocyte sera probably were hemolytic in type. No attempts were made to eliminate erythrocytes from the leukocyte antigen preparations, the antisera were unabsorbed, and it is very likely that erythrocyte antibody was present in each antiserum. The type of anemia which developed was thought to represent a temporary passive type of acquired hemolytic anemia. There was no relationship between the type of antisera administered and the severity of the anemias which developed.

In these studies it is presumed that a heteroimmune granulocyte antibody was produced in rabbits in response to the antigenic stimulus of guinea pig granulocyte cytoplasmic components. This antibody was capable of inducing severe granulocytopenia in guinea pigs in a fashion similar to that observed with the use of intact granulocytes[3, 9-13] and suggested by previous observations in human agranulocytic and leukopenic states.[26-38]

SUMMARY

1. Antisera to particulate fractions of guinea pig splenic cells and peritoneal exudates were prepared in rabbits.

2. Hematological observations were made in guinea pigs following a single intraperitoneal injection of heteroimmune leukocyte fraction antisera.

3. A significant and persistent granulocytopenia was induced in guinea pigs following the intrapertioneal injection of heteroimmune granulocytic cytoplasmic antiserum.

4. The response to the administration of granulocytic nuclear antiserum was much less pronounced.

5. Immature lymphocytes appeared in the peripheral blood of guinea pigs in response to the administration of splenic-cell nuclear antiserum.

6. A striking increase in the number of circulating granulocytes was observed following the injection of splenic-cell cytoplasmic antiserum.

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LEUKOCYTE RESPONSE TO GRANULOCYTE FRACTION ANTISERA

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IN THIS study an attempt has been made to compare the antigenic activity of subfractions of granulocyte cytoplasm. From guinea pig granulocytes the mitochondria, microsome, and supernatant protein fractions were obtained and injected into normal rabbits. The rabbit antisera produced were tested both for their leukocyte agglutinating capacity and for their ability to induce granulocytopenia in vivo.

It has been demonstrated that when guinea pig polymorphonuclear leukocytes are injected into rabbits, potent antisera develop which are capable of producing leukocyte agglutination and lysis in the test tube and profound leukopenia when administered to normal guinea pigs.[1-6] It appears from previous studies that the antigenic properties of guinea pig granulocytes reside principally in the cytoplasmic portion of the cell.[7] However, that portion of the cytoplasmic fraction which is most antigenic has not been established.

MATERIALS AND METHODS

Preparation of Leukocyte Fractions.—Peritoneal exudates were prepared in 20 guinea pigs by the method of Martin and associates.[8] A total of 2.9 ml. of packed leukocytes (about 95 per cent granulocytes) was recovered from these animals. The cytoplasmic portion of the leukocytes was recovered according to the technique previously described.[7] The nuclear sediment was discarded. The cytoplasmic fraction was separated further on a Spinco ultracentrifuge according to the technique of Umbreit, Burris, and Stauffer[9] for liver cell fractionation. Three granulocyte cytoplasmic fractions were recovered and these subsequently were used for the immunization of rabbits. The mitochondrial and the microsomal fractions were suspended separately in 14 ml. of 0.88 M sucrose solution and frozen until used. The final supernatant solution was frozen and used for immunization without further dilution. The protein content of the mitochondrial antigen was 40 mg. per cent, while that of the microsomal and final supernatant antigens were too small to be measured by the micro-Kjeldahl digestion technique used. Each fraction was used to immunize 2 male albino rabbits. These animals were injected intravenously with one of the three fractions at three- to four-day intervals for a total of five injections. The volume of antigen solution used for each injection of the mitochondrial and microsomal solutions was 1 ml., and for the final supernatant solution 2.5 ml. Five days after the last injection of antigen the animals were exsanguinated and the antisera were frozen in small aliquots.

In Vitro Test of Antisera.—An aliquot of each antiserum was inactivated in a water bath at 60° C. for thirty minutes. A 1:10 dilution of the inactivated antiserum in 0.85 per cent sodium chloride was prepared and serial saline dilutions were made to a dilution of

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1:2,560. Fresh leukocytes again were obtained from peritoneal exudates of normal guinea pigs, and a saline suspension containing 36,000 cells per cubic millimeter was used as the antigen. To 0.1 ml. of each dilution of antiserum in a 9 by 70 mm. test tube, 0.05 ml. of the leukocyte antigen was added. The tubes were mixed, allowed to stand at room temperature for two hours, centrifuged at low speed for one minute, and examined for macroscopic agglutination.

In Vivo Test of Antisera.—The ability of the antisera to produce granulocytopenia in normal guinea pigs was tested in a manner similar to that previously described.[7] Following the intraperitoneal injection of 2 ml. of each antiserum to normal guinea pigs weighing from 250 to 400 grams, the total and differential leukocyte response was studied at frequent intervals over a period of seven days. The effects of each of the 6 antisera were studied in from 4 to 6 guinea pigs. The serum from 2 normal rabbits was administered to a group of 10 normal guinea pigs in order to observe the nonspecific effects of the heterologous serum.

RESULTS

Antisera produced by the injection of both the mitochondrial and the microsomal fractions of guinea pig granulocyte cytoplasm exhibited marked in vitro agglutination of guinea pig leukocytes, but the antisera produced against

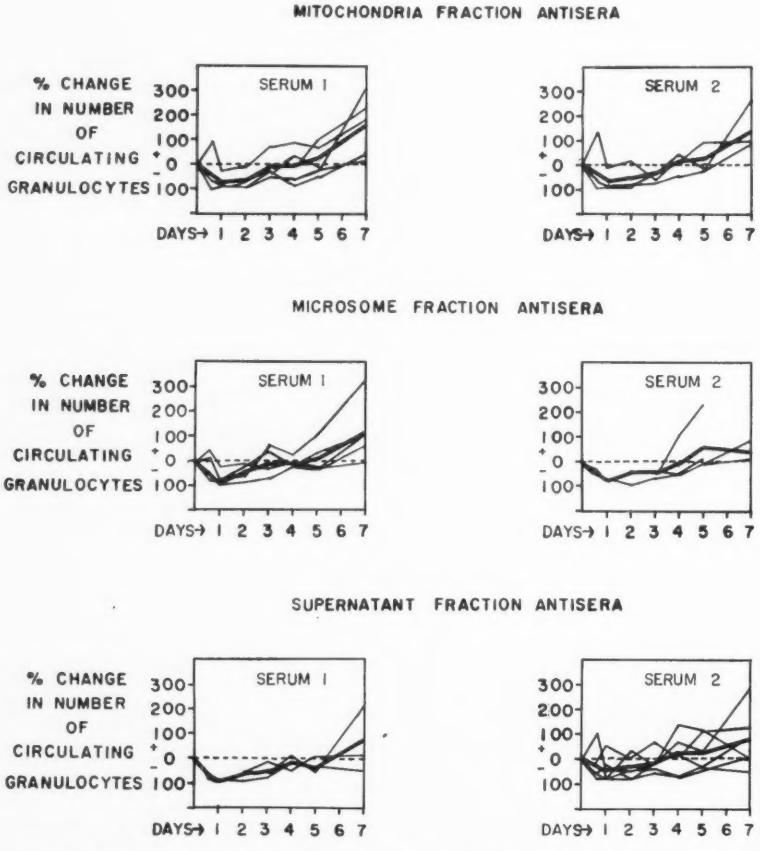

Fig. 1.—The heavy line in each graph represents the average percentage change in number of circulating granulocytes in normal guinea pigs following the administration of cytoplasmic fraction antisera. The thin lines represent the response of individual animals.

the final supernatant solution had only a minimal effect (Table I). Similar studies on normal rabbit serum demonstrated definite clumping of guinea pig granulocytes when mixed with undiluted serum, but no agglutination in a 1:10 saline dilution (Table I). Each of the plasma fraction antisera administered to normal guinea pigs produced a significant granulocytopenia of about the same duration and magnitude (Fig. 1). On the other hand, the results obtained following the injection of all experimental antisera were distinctly different from those obtained with normal rabbit sera (Fig. 2). There were some individual variations in response, and, in general, the guinea pigs with low initial leukocyte counts developed more marked granulocytopenia after injection of antisera than did the animals with high initial leukocyte counts. The mean response of the three cytoplasmic fraction antisera is compared with that of normal rabbit sera in Fig. 3.

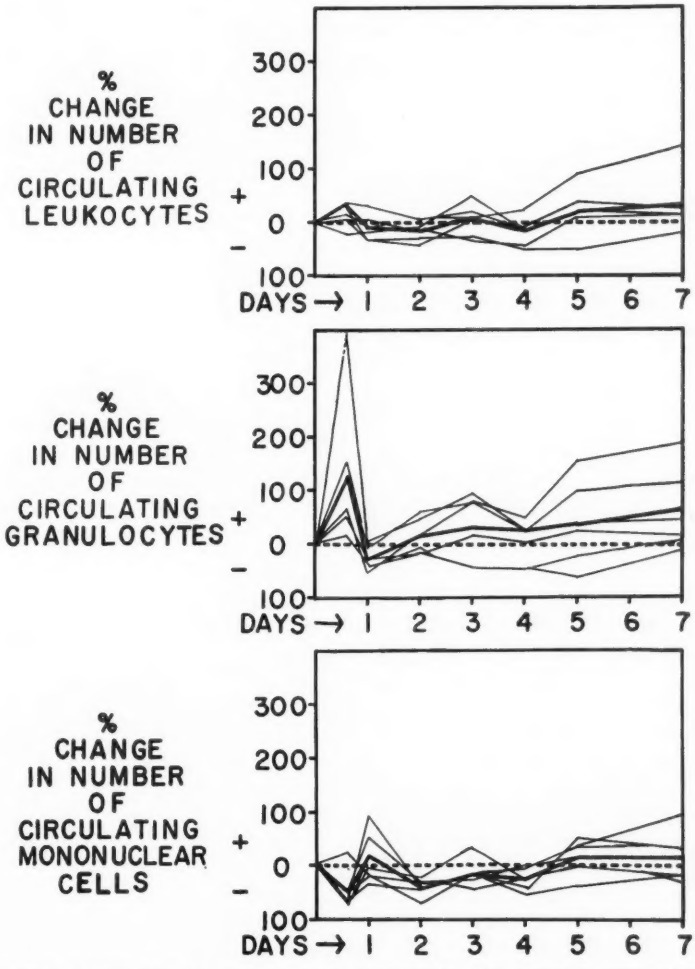

Fig. 2.—The heavy line in each graph represents the average percentage change in number of circulating leukocytes in normal guinea pigs following the administration of normal rabbit serum. The thin lines represent the response of individual animals.

TABLE I. GRANULOCYTE AGGLUTININ TITERS TO GRANULOCYTE CYTOPLASMIC ANTISERA

TYPE OF SERUM		SALINE DILUTIONS OF INACTIVATED ANTISERA									
		1:1	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Mitochondria	#1	++++	++++	++++	++++	++++	++++	++	±	0	0
Mitochondria	#2	++++	++++	++++	++++	++++	++	+	±	0	0
Microsome	#1	++++	++++	++++	++++	+++	+	±	0	0	0
Microsome	#2	++++	++++	++++	++++	++++	+++	+++	++	±	0
Supernatant	#1	+++	±	±	0	0	0	0	0	0	0
Supernatant	#2	++++	++++	++	+	±	0	0	0	0	0
Normal		+	±	0	0	0	0	0	0	0	0

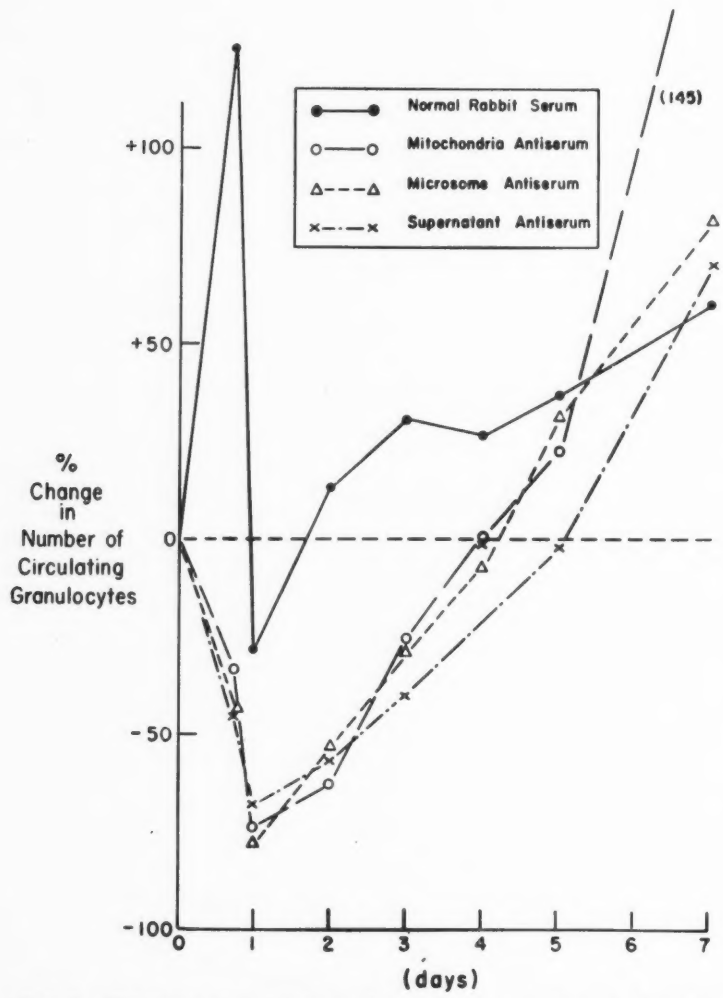

Fig. 3.—The average percentage change in the number of circulating granulocytes for each group of guinea pigs following the injection of cytoplasmic fraction antiserum is shown by the broken lines. The solid line represents the average percentage change in number of circulating granulocytes for all normal guinea pigs given normal rabbit serum.

DISCUSSION

The results of these studies do not suggest that the antigenic properties of leukocytes are localized in any single portion of the cytoplasm. The in vivo results obtained with the three cytoplasmic fraction antisera were comparable in magnitude, and when compared on a weight basis the results suggest that the microsome and final supernatant fractions are better sources of antigen than is the mitochondria fraction. These results, however, must be considered strictly semiquantitative since the precise protein content of the antigens was not known. It is also of interest to note that the magnitude of the granulocytopenia which occurred following the administration of cytoplasmic fraction antisera was closely comparable to that obtained when whole cytoplasmic fraction antisera were used.[7] Others have indicated that the mitochondrial portion of the mouse leukemic lymphocyte is the major source of leukocyte antigen,[10, 11] but in our studies with normal granulocytes we were unable to obtain this degree of antigenic localization. The interpretation of our results depends upon the efficacy of our separation of the cytoplasm into its component parts. Although the mitochondria and microsomes may have been poorly separated, it is highly improbable that any particle of mitochondrial size remained in the final supernatant solution. The profound antigenic properties of the final supernatant solution indicate, therefore, that portions of the leukocyte other than the mitochondria are highly antigenic.

The in vitro agglutination studies, in general, agreed fairly well with the in vivo granulocytopenic responses. However, the leukocyte agglutination titers of the final supernatant antisera were low (Table I) while their in vivo effects were comparable with those of the other types of antisera. The explanation for this lack of correlation is not clear. It is possible that the lytic properties of leukocyte antisera are independent of their agglutinating capacity, an effect comparable with the disparity between erythrocyte agglutinins and hemolysins.

No attempt was made in these studies to eliminate nonspecific effects of antibodies produced in the rabbit against other guinea pig proteins. Previous studies, however, have indicated that the leukopenic effect of heterologous leukocyte antisera is a specific response[12] and probably is due to antibody produced against leukocytes. In any event, heterologous leukocyte antisera do contain factors which are capable of producing profound leukopenia in vivo and leukocyte destruction, agglutination, and complement fixation in vitro.

It may be of considerable practical value to know which portion of the leukocyte is most antigenic. A purified granulocyte antigen of this type might be of greater value than intact cells in studies of leukocyte immune mechanisms. By the elimination of nonantigenic proteins of leukocytes it might be possible to produce pure drug-antigen complexes capable of inducing experimental autoimmune granulocytopenia. Animal studies of this type should be of importance in elucidating the mechanism of drug-induced leukopenias in man.

SUMMARY AND CONCLUSION

The cytoplasm of guinea pig granulocytes was fractionated into mitochondria, microsomes, and supernatant protein. Rabbit antisera prepared against

each of these fractions were tested for their in vitro agglutination and in vivo granulocytopenia effects. From this study it is concluded that all three fractions have appreciable antigenic properties.

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A STAIN FOR GRANULES IN NORMAL HUMAN ERYTHROCYTES

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IT IS possible to demonstrate the presence of granules in normal human erythrocytes with constant regularity by a method similar to the one used in the past for staining peroxidase granules in the white blood cells. This can be accomplished by modifying the old benzidine-peroxide technique[1, 2] by decreasing the pH of the reagent and increasing the concentration of ethyl alcohol. The latter serves to slow down the rate of reaction markedly, so that a very slow deposit of stain is produced in nine to twelve minutes that is limited to the corpuscles themselves.

The stain is made by mixing two specially prepared solutions of benzidine and hydrogen peroxide as follows:

The first solution consists of 900 mg. of recrystallized benzidine dissolved in 60 c.c. of 70 per cent ethyl alcohol to which 0.3 c.c. of glacial acetic acid is added. A trial sample is then made by taking 10 c.c. of this reagent and further diluting it to 25 c.c. with 70 per cent ethyl alcohol. This is labeled "Solution A."

The second solution consists of 40 c.c. of 70 per cent ethyl alcohol, 9 c.c. distilled water, and 1 c.c. of 3 per cent hydrogen peroxide (U.S.P.). This solution is labeled "Solution B."

PROCEDURE

A direct blood smear, or a smear of oxalated blood no more than seven days old, may be used. The smears should be uniform, very thin, and free from rouleaux.

In a small well, such as the cap of a small bottle, mix Solution A–12 drops and Solution B–3 drops.

Fix the blood smear for at least five minutes in 70 per cent ethyl alcohol, dry with tissue paper, and divide the slide into uniform squares of 1 sq. cm. each with a wax pencil.

Place 3 drops of the mixture of Solutions A and B on one square, and every three minutes add another drop, at the same time staining another square with 3 drops, until four squares have been stained for twelve, nine, six, and three minutes, respectively. Rinse off the staining solution with water, dry, and examine under the oil immersion lens.

A properly stained slide should show a faint blue tint at the end of nine to twelve minutes, when the dried slide is viewed on a white background. The stain is an extremely sensitive one and is not as satisfactory with a freshly prepared solution. It seems that it gives better results on standing for a few days after it is prepared. The fresh stain has a tendency to produce fine amorphous granules of blue or black.

A good stain will show the white cells with large blue granules in all the squares, and they will be deepest in the twelve-minute stain. The red blood cells will appear a uniform yellow in the three- and six-minute stains, but the nine- and twelve-minute stains will show the presence of well-defined blue or blue-black rods and granules.

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Fig. 1 shows a typical light stain. Rods predominate, they may be straight or comma-shaped, and the cell outline is distinct.

Fig. 2 shows a heavier stain. The rods have become so numerous that the cell outlines are fairly obliterated and take on a blue color. Some of the rods will show clubbing at either end and some will be curved. In some smears granules will predominate, but in the majority the rods will prevail, depending upon very slight changes in technique.

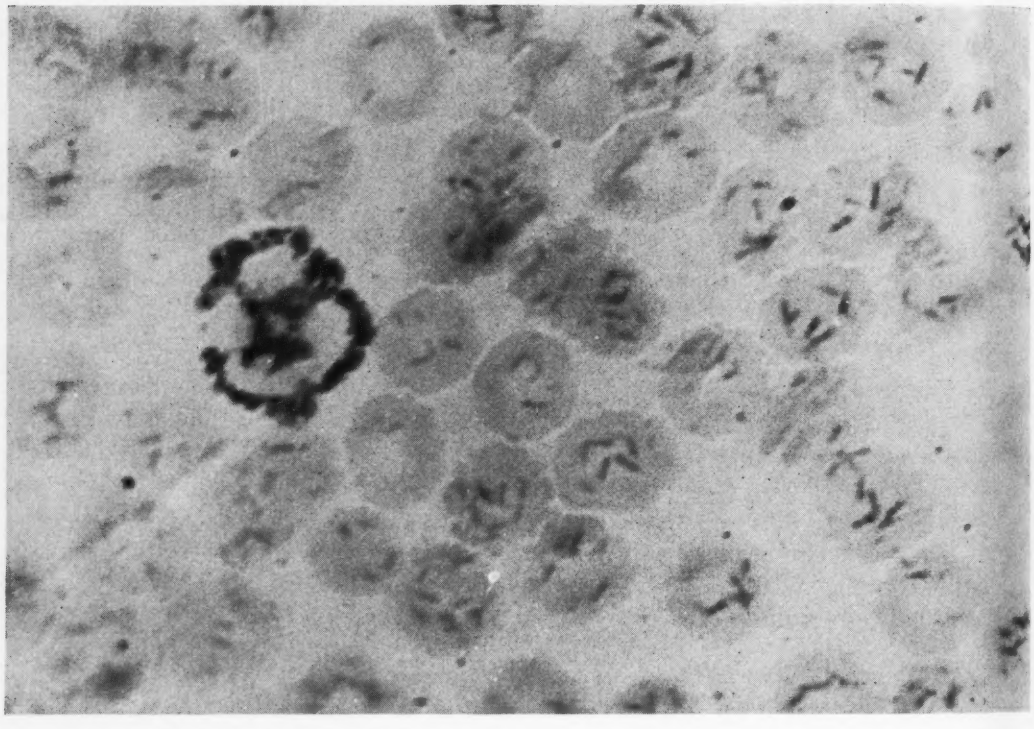

Fig. 1.—A light stain. The white blood cells show deep blue granules in the cytoplasm. The red blood cells show a moderate number of rods and granules.

If none of the four squares shows the granules, repeat the stain, using a mixture of 4 or 6 drops of the peroxide reagent to 12 drops of the benzidine reagent. If the red cells come out a uniform blue without rods or granules, reduce the peroxide reagent to 1 or 2 drops.

The stain is intensified by:

1. A thinner smear where there are fewer red blood cells per field.
2. Adding a drop of dilute acetic acid to the stain.
3. Adding a drop of peroxide reagent (Solution B) to the stain.
4. Increasing the time of staining.

The stain is diminished by:

1. A thicker smear where there are more red blood cells per field.
2. Adding a drop of dilute sodium bicarbonate solution to the stain.
3. Decreasing by one drop the amount of peroxide reagent.
4. Decreasing the time of staining.

The stain of the granules in the white blood cells can be markedly suppressed by doubling the concentration of the peroxide reagent, without similarly suppressing the stain of the red cells.

The stain of the red cells comes out the same, whether or not the slide is first fixed in 70 per cent ethyl alcohol for five to fifteen minutes.

An aqueous solution of crystalline catalase in distilled water smeared on a slide, dried, and stained in the same manner gave similar blue granules.

Whether the staining effect described is due to catalase, peroxidase, or some other oxidase enzyme present in the erythrocytes remains to be established.

Fig. 2.—A deeper stain. The outline of the red blood cells is obliterated. The rods and granules are larger, more numerous, and show a deep blue color.

SUMMARY

An extremely sensitive stain for granules in normal human erythrocytes is presented. It is based on the old benzidine-peroxide technique used in the past for staining granules in the white blood cells but modified to suppress the effect on the latter and to bring out granules in the red cells instead.

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THE DETECTION OF A LATENT ANTIBODY IN ABO HEMOLYTIC DISEASE

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IT WAS Witebsky[1] who called attention to the nature of immune A_1 antibody associated with ABO hemolytic disease of the newborn. His observations and techniques have formed a basis for subsequent extensive clinical studies[2-5] of heterospecific pregnancies which have shown that the immune type anti-A antibody is usually present in high titer in the maternal serum. This, too, has been our experience, and we wish to report an exception and an additional unusual antibody of a latent nature. The antibody occurred in two mothers who gave birth to nonicteric infants who were later shown to have hyperbilirubinemia necessitating exchange transfusion.

Our serologic study in these two cases was further stimulated by a report by Garmier,[6] who observed a case of a stillborn infant whose group O mother (father group A) showed very little immune anti-A prior to delivery but who subsequently revealed very high titers of this antibody. In addition, during our recent study of blood groups in habitual abortion,[7] we occasionally encountered heterospecific matings in which the mother failed to reveal immune anti-A or B antibody. In the past decade, several papers have appeared analyzing the significance of hemolytic disease and ABO incompatibility.[8-10] All the authors agree that severe hemolytic disease due to this cause is relatively infrequent but that its importance should be kept in mind when hyperbilirubinemia is noted in the infant. Our cases supplement the observations of Witebsky and, in addition, emphasize the occurrence of a latent immune anti-A antibody.

METHODS

Family Study.—The two families, diagramatically shown in Fig. 1, were referred to us because of a history of neonatal hemolytic deaths and stillborn fetuses. The L. R. family was studied more extensively because they presented themselves earlier in the prenatal stage. Upon each visit blood was obtained and the inactivated serum analyzed for isoagglutinins and immune antibodies, as described following. The serum samples were then saved and stored, frozen at -15° C. without the addition of A and B substances. These specimens were re-examined after storage, as indicated in Table I.

Table II, a chart of laboratory results, indicates the degree of hemolytic disease of the two newborn infants. The pediatricians caring for these infants considered their condition to be typical hemolytic disease of the newborn.

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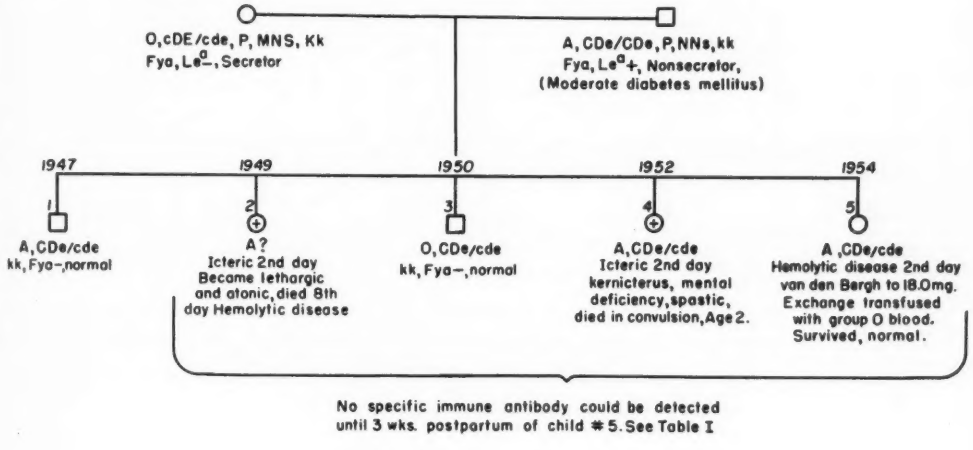
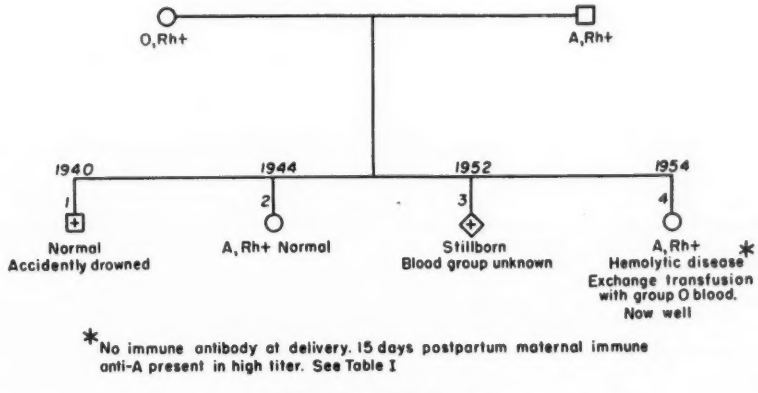
SEROLOGIC METHODS

1. *Isoagglutinin Titer.*—The standard serial twofold dilution titration technique using 0.9 per cent saline solution and dry transfer pipettes was adhered to. An equal portion of 2 per cent A_1 cells in 0.9 per cent saline solution was added to each tube. Centrifugation for one minute at 2,000 r.p.m. and recording of the last 1 plus reaction obtained upon gentle agitation determined the end point.

2. *"Immune" Anti-A (Witebsky Test).*—The serum to be tested was neutralized by the addition of two parts of commercial A and B substance to one part of inactivated (56° C. for thirty minutes) serum. The mixture was permitted to stand at room temperature for ten to fifteen minutes and then tested against a 2 per cent saline suspension of A_1 cells for completeness of neutralization. If neutralization was incomplete, three parts of A and B substance was added in place of two. In neither case was a greater quantity of A and B substance required to effect neutralization.



CASE 1. THE L.R. FAMILY



CASE 2 The S.B. Family

Fig. 1.—Pedigree of maternal latent antibody cases.

The neutralized serum was titrated in inactivated, pretested (nonspecific agglutinin free) AB serum by the twofold dilution method. Equal portions of 2 per cent A_1 red cells suspended in AB serum were added and the tubes incubated at 37° C. for one hour. The

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TABLE I. SEROLOGIC CHARACTERISTICS OF MATERNAL LATENT IMMUNE ANTI-A ANTIBODY

	MATERNAL SEROLOGIC TITERS									INFANT STUDY		
					WITEBSKY TEST		INDIRECT COOMBS TEST		INDIRECT ELUTION TEST			
CASE	MATERNAL BLOOD GROUP	DATE	ANTI-ISO-AGGLUTININ TITER	IMMEDIATE	FROZEN STORAGE	IMMEDIATE	FROZEN STORAGE	IMMEDIATE	FROZEN STORAGE	DIRECT COOMBS TEST	DIRECT ELUTION TEST	CLINICAL CONDITION
I. L. R.	O Rh +	Oct., 1953	1:256	0	0	0	+	0	+			Normal female infant; blood group A_1 Rh+; icteric in 24 hours and exchanged with type 0 blood; infant improved and check-up at six months revealed normal development (Fig. 1)
		Jan., 1954	1:256	0	0	0	1:384	0	+			
		Feb., 1954	1:256	0	0	0	1:384	0	+			
		March, 1954	1:256	0	0	0	1:192	0	+			
	Delivery date	April 21, 1954	1:1,024	0	0	0	+			0	+	
		May 6, 1954	1:1,024	1:6,000	1:6,000	+	1:12	+	+			
		June, 1954	1:1,024	1:6,000	1:6,000	+	1:192	+	+			
II. S. B.	O Rh +											Infant born nonicteric; blood group A_1 Rh+; required exchange transfusion in 24 hours because of hyperbilirubinemia; following exchange, infant less icteric and uneventful development
	Delivery date	May 9, 1954	1:1,024	0	0	0	+	0	+			
		May 24, 1954	1:1,024	1:3,000	Not done	+	1:768	+	+			

0=negative test; +=positive test (2 to 4 + reaction).

TABLE II. LABORATORY AND CLINICAL DATA OF THE TWO CASES STUDIED

	DATE	VAN DEN BERGH DIRECT	VAN DEN BERGH TOTAL	HGB.	HCT.	BLOOD SMEARS	CLINICAL REMARKS
Case I							
Baby R	April 21, 1954 (Cord)	0.25	5.8				Nonicteric
Group A	April 21, 1954	0.6	9.2	13.0	34	Polychromatophilia Normoblasts 10	
	April 22, 1954	1.9	14.4	12.0	30	Normoblasts	Clinical icterus
	April 23, 1954	0.8	18.0	13.0	37		Lethargic, large liver
	April 24, 1954			13.3	36	No normoblasts	Replacement transfusion
	April 27, 1954	1.5	18.0				
	April 28, 1954			15.0		O.K.	
	April 30, 1954			15.6		O.K.	Icterus receding
Case II							
Baby B	May 9, 1954 (Cord)	0.5	2.25				Nonicteric
Group A	May 10, 1954	0.75	7.2	15.0	42	Spherocytosis	Clinical icterus Liver and spleen large
	May 10, 1954	0.8	17.8	16.0	44	Metamyelocytes Polychromatophilia	Replacement transfusion
	May 11, 1954	0.7	20.8	14.0	37	Normoblasts	
	May 12, 1954	0.6	16.2	14.6	43		
	May 15, 1954		9.1				Icterus fading

tubes were centrifugated at 2,000 r.p.m. for two minutes and the reactions examined after gentle agitation. A fine but distinctly granular agglutination was considered a 1 plus reading and the end point of the titration.

3. *Indirect Coombs Test.*—The inactivated and isoagglutinin neutralized serum was used to sensitize A_1 cells at 37° C. for one hour. Equal portions of 2 per cent cells in saline and serum were used for this purpose. The sensitized cells were washed three times with tubefuls of 0.9 per cent saline, and, after the final washing, the cells were resuspended to make a 2 per cent suspension. To two drops of this cell suspension, two drops of antihuman globulin serum were added, and the mixture was centrifuged at 500 r.p.m. for one minute. The reactions were examined after gentle agitation of the tubes, a 2 or 3 plus reaction being considered positive. Weaker reactions were confirmed or rejected after rewashing the cells and repeating the antiglobulin test.

4. *Indirect Elution Test.*—Five-tenths millimeter of maternal serum was added to 1.0 ml. of commercial A and B substance, and, after this serum stood for ten minutes at room temperature, it was tested against saline-suspended A_1 cells for completeness of neutralization. To 0.5 ml. of this neutralized serum was added 0.5 ml. of washed, packed A_1 cells in saline solution. Similar tubes were set up using A_2 cells and group O cells as controls. The serum-cell mixtures were incubated in a water bath for one hour at 37° C. The cells of both tubes were washed three times with cold 0.9 per cent saline solution, resuspended in 0.5 ml. of saline solution, and heated at 56° C. for ten minutes. The incubated tubes were then centrifuged at 2,500 r.p.m. for two minutes and the supernatant eluate removed. Two drops of this eluate were added to 2 drops of 2 per cent saline-suspended, fresh A_1 cells, and finally 4 drops of 10 per cent PVP[11] (polyvinylpyrrolidone) were added and the mixture centrifuged at 2,000 r.p.m. for one minute. Agglutination of the cells after gentle agitation of the tubes indicates the presence of immune type anti-A in the maternal serum. The control tubes were, of course, negative.

5. *Direct Elution Test.*—Clot-free fetal cord blood was centrifuged to obtain 0.5 ml. of packed red cells. These cells were then washed three times and resuspended in 0.5 ml.

of saline. This cell suspension was then heated in a water bath at 56° C. for ten minutes followed by centrifugation at 2,000 r.p.m. for two minutes and removal of the supernatant eluate. Two drops of saline-suspended A_1 cells, two drops of eluate, and 4 drops of 10 per cent PVP[11] were added, and, after mixing, the tubes were centrifuged at 1,500 r.p.m. for one minute. Parallel tubes in which group O cells and A_2 cells were employed served as controls. After centrifugation, the tubes were gently agitated and the degree of agglutination recorded. A 2 to 4 plus reaction was considered indicative of sensitization of the cells with anti-A antibody. Control tubes were negative.

RESULTS

Fig. 1 illustrates the families in which the mother in each case demonstrated an immune anti-A only after three weeks post partum. Previous infant mortality due to hemolytic disease or unknown cause (stillborn) was observed in each family. The recently delivered infant was born uneventfully in each case and appeared normal until the second day, when jaundice was clinically observed. Antibodies for Rh or rarer blood factors could not be identified in either mother. Each infant was given a replacement transfusion of group O blood and showed immediate and continued benefit. The total serum bilirubin did not rise over 18 mg. and then declined to normal. At the time of birth, antibodies could not be detected, but, because of the heterospecific incompatibility, group O blood was given empirically.

Table I shows the sequence of antibody development. In the isoagglutinin column, the saline titer is noted to be high, since by our technique a normal titer of isoagglutinins rarely exceeds 1:128. At the time of delivery (April 21, 1954), a sudden rise to 1:1,024 occurred in the serum of Case 1. Throughout the prenatal study, the immune anti-A was absent by all tests. In each case a marked rise in immune anti-A occurred three weeks post partum and the indirect Coombs test became positive, as did the elution reaction. The elution tests were particularly convincing because the control A_2 and O cell controls reacted negatively, thus affirming the specificity of immune anti-A_1.

Re-examination of the frozen, stored samples produced the surprising finding of immune anti-A by the Coombs and elution tests but not by the Witebsky neutralization technique. The length of storage time varied, of course, with each specimen, but all had been frozen at least one month. The positive maternal Coombs reaction was not equivocal, and a panel of control O cells was negative.

DISCUSSION

The maternal serum finding of an antibody which during gestation was undetectable by all known methods is of considerable theoretical interest. In vitro it is well known that isoagglutinins anti-A or B may be neutralized by their respective A or B substance and that later, after storage, the isoagglutinin reappears. However, the serums which we stored contained no A or B substance. A and B substances were employed only in the small aliquots of serum used to perform the Witebsky and Coombs tests. Furthermore, with the exception of the direct elution test, the infant's serum and cells were negative for antibodies. The development of progressive hemolytic disease of the infant, the demon-

stration of anti-A_1 antibody by direct elution, and the subsequent high titer of maternal immune anti-A_1 all testify to the specificity of ABO hemolytic disease. What, then, is the explanation of this hidden or latent maternal antibody?

One possible explanation might be that a rare blood group was responsible for the hyperbilirubinemia of the infant. With this possibility in mind, we set up repeated panels of group O cells, with entirely negative results. It was concluded, therefore, that we were dealing with an unusual anti-A agglutinin of high potency that was inhibited during the time of gestation.

Since the maternal serum collected after delivery gave a strong Witebsky's test and an equally strong indirect Coombs titer, the behavior at this time simulated that of Rh antibodies, as was also observed by Hubinont.[15] In addition, recent studies have convinced us that the most important pathogenic antibody is that demonstrated by the Coombs test.[12, 13] Hill, Haberman, and Jones[14] have referred to this as a cryptogglutinin or third order type, in distinction to the blocking or conglutinin type. The antibody that we have demonstrated fits this third order type. We were able to clearly separate the two antibodies upon reexamination of the serums as demonstrated in Table I.

The next question which confronted us was that of the source and nature of the inhibiting substance which completely hid the antibodies before parturition. Several possibilities must be considered: (1) the infant produced an A-like substance which neutralized or absorbed the maternal antibodies but which was ineffectual after delivery; (2) that the mother, early in pregnancy, manufactured an inhibiting substance which counteracted her own anti-A antibody; or (3) that perhaps a neutralizing placental product was the source of the observed antibody suppression substance. Experiments are now being devised to test these hypotheses. Whatever the source, we know that the substance must be quite labile since, upon storage, it degenerates. Tovey[16] now favors the first possibility as being most likely.

A few practical recommendations should be mentioned in connection with this study and our general experience with ABO hemolytic disease:

1. Obstetricians should request ABO grouping as well as Rh typing on expectant mothers.

2. Group O mothers[4] as well as Rh negative mothers should be regarded as potential hemolytic disease problems, and their husbands' blood groups should be determined.

3. A direct cord blood elution test for the presence of anti-A and anti-B is recommended as a supplementary procedure in the elucidation of problems involving these antibodies. This applies especially when the Witebsky neutralization and the Coombs tests are negative.

4. Replacement transfusion with group O blood is effective in controlling ABO hemolytic disease of the newborn.

Our two cases are supplementary to that described by Garmier,[6] and undoubtedly the antibodies were of a similar latent nature. We wish to emphasize the unusual behavior of this anti-A antibody and the value of the elution test in its demonstration. The direct Coombs test did not reveal the antibody.

SUMMARY

Although most cases of hemolytic disease due to ABO heterospecific pregnancy demonstrate immune anti-A_1 antibodies, we have encountered two in which only latent anti-A was present.

In these cases the direct elution test on the cord blood is of value.

ABO heterospecific matings in which the mother is group O deserve antibody analysis to forewarn the physician of possible hemolytic disease and its sequelae.

The demonstration of a latent antibody suggests again the possibility of a labile protective substance in the maternal and fetal circulation.

We wish to thank Doctor Milo C. Moody of Spanish Fork, Utah, for referring Case 1 to us and furnishing the important historical facts and physical findings.

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A QUANTITATIVE MODIFICATION OF THE "TUBELESS" GASTRIC ANALYSIS

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THIS preliminary report describes our experiences with a quantitative interpretation of the "tubeless" gastric analysis. Segal and associates[1] described this method of measuring gastric acidity first in 1950, and reported further experience with the test in two other papers.[2, 3] Many reports from other centers have confirmed and extended their original observation.

The new test employs a cation-exchange resin commercially available as Amberlite XE-96. In the test resin* the cation-exchange hydrogen ion is replaced by a quinium radical (Fig. 1). In the pH range of free hydrochloric acid, pH 3 to 4 or less, the hydrogen ions of gastric juice displace the quinium ions, which then form quinine hydrochloride.

The quinine is absorbed rapidly from the stomach and is excreted in the urine. The urinary quinine can be estimated accurately by comparing its ultraviolet fluorescence with standard quinine solutions.

Reports to date have compared the "tube" and "tubeless" methods of gastric analysis in large groups of normal and abnormal subjects.[3, 4, 5, 7] The correlation between the two methods is uniformly good in the achlorhydric group but rather poor in those cases with free hydrochloric acid. All observers agree that no quantitative relationship can be shown between the amount of quinine excreted in the urine and the acidity of the aspirated gastric juice measured as "clinical units" or as pH.

Our experience seems to resolve the apparent inconsistencies in the two tests. A brief review of current knowledge about the formation of HCl will help to clarify our results.

Physiology of HCl Formation.—Hydrochloric acid is produced only in the parietal cells of the gastric mucosal glands. When first demonstrable, the HCl molecule is isotonic with blood plasma, and its hydrogen-ion concentration is 159 meq. per liter.[10] It is acknowledged that HCl is always produced in the same concentration by the parietal cells.[11] The final concentration of free acid in gastric juice, however, is never much higher than 50 meq. per liter. This means that dilution of the original HCl is produced by a variety of other substances. Some are swallowed, like the test meal and saliva, others are secreted,

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*Supplied by E. R. Squibb & Sons as "Diagnex."

like mucin and pepsin in the stomach, and there is often some pyloric reflux. It is apparent that "hyperacidity," measured in the usual way, can be reached either by secreting more acid or by less dilution.

Gastric juice usually is tested for acid by titrating a measured volume with N/10 NaOH solution.[12] An end point of pH 3 to 4 is reached first, using Topfer's reagent as the indicator. The number of milliliters required to neutralize 100 ml. of gastric juice to this end point defines "clinical units of free acid." The additional volume required to reach an end point of pH 7, using phenol red as the indicator, gives units of "combined" acid.

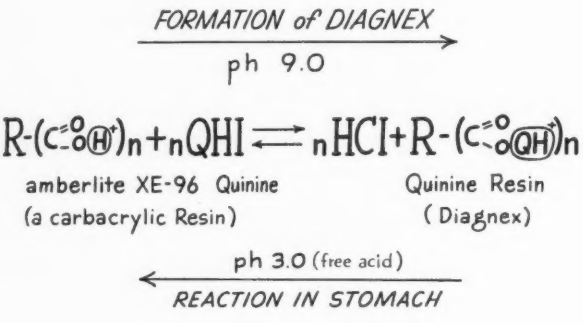

Fig. 1.—Cation exchange resins.

Consideration of the ion exchange reaction suggested that the amount of quinine exchanged should depend upon the total number of available hydrogen ions in the gastric juice, rather than upon their concentration alone. In other words, if one measured not only the concentration but also the volume of gastric juice aspirated in the test period, it should correspond to the amount of quinine in the urine. The figures obtained in our studies seem to support this premise.

METHODS

In the past year, 101 patients have been examined with both the quinine test and a conventional gastric analysis. If no free acid was present in the fasting aspirated sample, 0.2 mg. of histamine was injected subcutaneously.

The technique we used in the ion exchange resin test is that printed on the commercial "Diagnex" package. Briefly, the patient in a fasting state receives a 250 mg. capsule of caffeine sodium benzoate as a gastric stimulant. An hour later, after collecting a control sample of urine, the patient swallows 2 Gm. of the resin granules, equivalent to 36 mg. of quinine. The urine excreted during the first and second hours after swallowing the resin is collected. Quinine is extracted from the urine by the ether-H_2SO_4 method used by most investigators.[9] The ultraviolet fluorescence of the urine specimens is then compared with standard quinine solutions, in a Fisher fluorophotometer.

RESULTS

The data obtained in this series are represented in Fig. 2. Since it was suspected that the urinary quinine excretion might be related quantitatively to the total available hydrogen ions in the aspirated gastric juice, the latter

quantity had to be expressed as a single figure. This was accomplished by converting the volume and concentration of each aspirated specimen to milliliters of N/10 HCl. When the mean values of aspirated acid in this unit were plotted against the mean values of urinary quinine excretion, a straight-line relationship was evident, as illustrated in Fig. 2. This line could be represented by the equation:

$$X = \frac{(Y - 82)}{7.3}$$ where and

X = ml. N/10 HCl aspirated

Y = micrograms of quinine

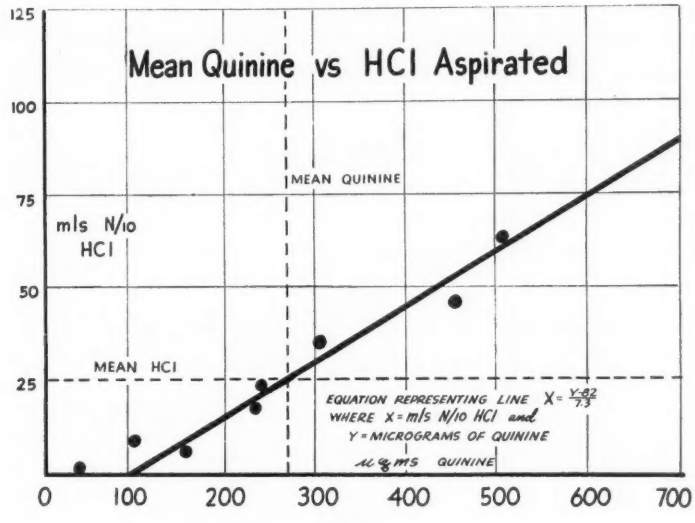

Fig. 2.

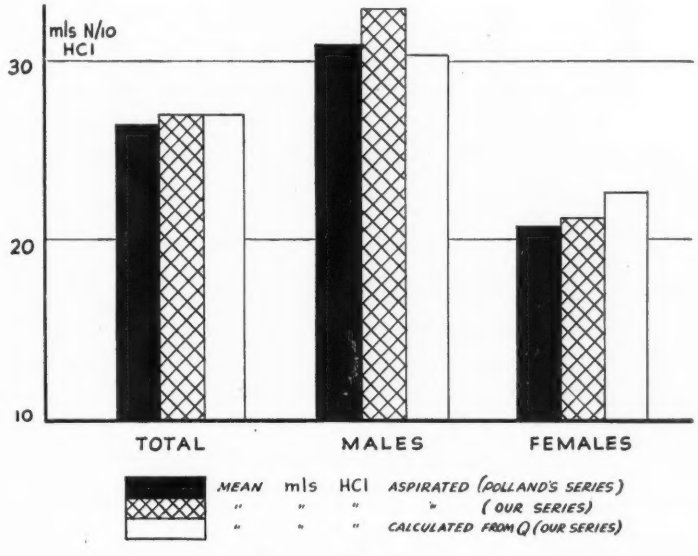

Fig. 3.

Evidently, then, a quantitative relationship between the two tests does exist. By applying this formula to the urinary quinine values, one can estimate the equivalent acid secretion expressed as milliliters of N/10 HCl acid. Statistical analysis of these figures indicates a correlation coefficient of 0.9.

It seemed worth while to compare the values obtained in our series with other similar groups of cases. Polland[13] has reported a large series of gastric analyses, giving both the concentration and the volume of gastric juice, and for comparison his values were transposed to milliliters of N/10 HCl.

Fig. 3 represents average acid values in milliliters of N/10 HCl obtained in Polland's series, compared with the aspirated values in our series, and the acid values in our series calculated from the urinary quinine. Within the statistical limitations of the diagram, there is good correlation among the three.

Fig. 4 represents a partial breakdown of our 101 cases, according to the type of disease present. In each of the columns, the values of acid aspirated and the values of acid calculated from the urine test are represented. Again, good correlation between the tests in the various disease groups seems to exist.

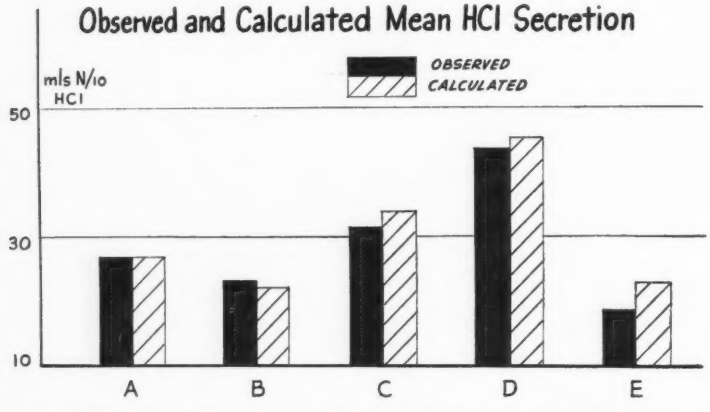

Fig. 4.—*A*, Total series, 101 patients; *B*, no organic gastrointestinal disease, 58 patients; *C*, organic gastrointestinal disease, 43 patients; *D*, peptic ulcer, 23 patients; *E*, miscellaneous disease, 20 patients.

DISCUSSION

In the achlorhydric group, our experience parallels previous reports. None of the patients with histamine achlorhydria by the tube method showed acid by the urine test. However, a few patients who had very little urinary quinine did show free acid by the tube method after histamine stimulation. Therefore, with use of the quinium method as a screening test, the negatives should be rechecked by doing either or both tests after histamine.

The main interest of this study is to extend the use of the resin test to the quantitative measurement of free HCl. It should aid in situations where the usual gastric analysis cannot be used or is inaccurate.

Some observations on the expense of the test may be of interest to laboratories considering its use. The cost of an instrument* for comparing ultraviolet fluorescence is the chief initial expense. A fluorophotometer may also be used for a variety of other comparison tests. Technician time required to process the specimens will average forty-five minutes per test. This approximates the time required to perform intubation, aspirations, and titration of specimens in conventional gastric analysis. The new test is certainly more popular with patients and should be no more expensive than the "tube" method, in routine use.

CONCLUSIONS

In 101 patients a simple quantitative correlation has been demonstrated between the two-hour urinary quinine excretion in the "Diagnex" test and the hydrochloric acid aspirated in the conventional gastric analysis. This should extend the value of the "tubeless" test beyond its present application as a screening test for achlorhydria and may enable it to replace the usual "tube" test in many situations.

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*This instrument may cost as little as $75.00 for a simple qualitative device, or as much as $600.00 for an accurate fluorophotometer.

COMPARISON OF VENOUS AND ARTERIAL HEMOGLOBIN CONCENTRATION IN MAN: RELATIONSHIP TO THE CALCULATION OF PER CENT OXYGEN SATURATION

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THERE is evidence that the hemoglobin concentration in man varies with age and sex,[1] muscular activity,[2] and time of day.[3] Peters and Van Slyke[4] state that there is a difference between hemoglobin concentration in venous and capillary blood and, in the presence of "moderate retardation of the peripheral circulation," between venous and arterial blood, but they give no data to support the latter statement. The per cent of oxygen saturation is the ratio of the oxygen content to the oxygen capacity. If it is true that the arterial and venous oxygen capacities in a given individual are different, arterial and venous oxygen saturations must be calculated on the basis of arterial and venous oxygen contents and capacities, respectively.

As there are practical advantages to the interchangeable use of venous and arterial capacities in the calculation of per cent oxygen saturation, the following data relevant to this problem are presented.

METHODS AND RESULTS

The oxygen capacity of arterial and venous blood was determined in twenty-one unselected patients, all men, in the process of various tests of cardiac and pulmonary function. All were at rest. Mild cardiac decompensatian was present in a few. Venous blood was obtained from an arm vein in fifteen instances, superior vena cava in one, right atrium in three, and pulmonary artery in two; and arterial blood was obtained from a femoral or brachial artery. Specimens were drawn not more than five minutes apart and were stored in ice. The blood was saturated with oxygen by rotation in an open tonometer in room air for thirty minutes, and its oxygen content was determined by the method of Van Slyke and Neill[5] with duplicate samples checking within 0.2 volume per cent.

RESULTS

The results are tabulated in Table I.

The range of the values for oxygen capacity is from 17.40 to 26.37 volumes per cent for arterial blood, and from 17.13 to 26.47 for venous. The means are 19.67 and 19.49, respectively. The standard deviation is 2.22 for the arterial values and 2.05 for the venous, while the corresponding standard errors of the mean are 0.48 and 0.45. Analysis by the "Student" t tests shows that the difference between these means could be expected to occur by chance alone about 80 per cent of the time ($p = 0.8$).

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TABLE I. ARTERIAL AND VENOUS OXYGEN CAPACITY IN TWENTY-ONE PATIENTS.

PATIENT	ARTERIAL		VENOUS		A-V O_2 CAP. DIFF. VOL. PER CENT
	O_2 CAP. VOL. PER CENT	HGB. GM. PER CENT	O_2 CAP. VOL. PER CENT	HGB. GM. PER CENT	
1	19.00	14.18	19.41	14.48	−0.41
2	19.83	14.80	19.27	14.38	+0.56
3	18.71	13.96	19.87	14.83	−1.16
4	19.30	14.40	18.14	13.54	+1.16
5	20.98	15.66	19.86	14.82	+1.12
6	18.00	13.43	17.49	13.05	+0.51
7	21.80	16.27	21.56	16.09	+0.24
8	23.34	17.42	22.49	16.78	+0.85
9	18.46	13.77	18.97	14.15	−0.51
10	26.37	19.68	26.47	19.75	−0.10
11	18.32	13.67	18.34	13.69	−0.02
12	21.06	15.71	20.52	15.31	−0.46
13	19.06	14.22	18.72	13.97	+0.34
14	17.68	13.19	17.48	13.04	+0.20
15	17.78	13.26	17.50	13.06	+0.28
16	17.88	13.34	17.94	13.39	−0.06
17	21.27	15.77	20.99	15.66	+0.28
18	17.40	12.99	17.13	12.78	+0.27
19	18.95	14.14	18.42	13.74	+0.53
20	18.90	14.10	19.72	14.71	−0.82
21	19.06	14.22	18.92	14.12	+0.14

Values have been corrected for physically dissolved oxygen. Hemoglobin obtained by dividing oxygen capacity by 1.34.

SUMMARY AND CONCLUSIONS

The relation of venous to arterial hemoglobin concentration as indicated by the oxygen-combining capacity has been studied in twenty-one men. Differences are very small and are statistically insignificant. It is felt that the interchangeable use of venous and arterial oxygen capacities is permissible in the calculation of venous and arterial per cent oxygen saturation.

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LABORATORY METHODS

CORRECTION OF ERRATIC REAGENT BLANKS IN DETERMINATION OF SERUM PROTEIN-BOUND IODINE

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FOR the past several years a large number of methods for and modifications of the chemical determination of protein-bound serum iodine has appeared in the literature. Chaney,[2] in his review of the techniques for protein-bound iodine, has an excellent summary of the various steps and methods for this determination. The present report has to do with an improvement in the alkaline ashing method of determination as reported by Salter and McKay,[4] Barker, Humphrey, and Soley,[1] and others.

It deals with two serious sources of error: (1) the use of Cl ion in the form of either NaCl or HCl; (2) the iodine impurity that is present in NaCl if it is used in the procedure.

Sandell and Kolthoff[5] found a marked enhancement of the catalytic effect of iodide on ceric-arsenite systems by high concentrations of chloride.

The effect of the chloride ion has been studied in subsequent papers, and in 1951 a report by Lein and Schwartz[3] appeared which stressed the need for chloride ion in this reaction to increase the sensitivity of the procedure and also to produce a straight-line relationship between the first order velocity constant and iodide concentration.

Our laboratory has been using the Barker, Humphrey, and Soley procedure[1] modified to include the sodium chloride as suggested previously with one additional step. The sodium chloride was dissolved in the 7N sulfuric acid, and the 2N HCl was titrated against the 4N sodium carbonate and one of the solutions was adjusted to make them equivalent. This was done in order to minimize the effect of chloride ions on the catalytic reaction of the ceric-arsenite system.

After this was done, many serum samples were determined over a period of several months with satisfactory results. However, because of erratic blanks, it was found necessary to redetermine some of the samples until duplicate results were obtained. Because the reagent blanks did not stay constant and at times were rather high, a study of the whole procedure was instituted.

It was found that the sodium chloride used, although chemically pure, had as much as 0.002 per cent iodide according to the analysis on the bottle. Consequently, using 100 mg. of sodium chloride per sample, as suggested by Lein and Schwartz,[3] means adding about 2 μg of iodide to the sample and reagent blanks. This would be equivalent to 200 μg per 100 ml. of serum, or roughly 100 times as much as is present as protein-bound iodine.

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Upon elimination of the sodium chloride and hydrochloric acid from the procedure we found that the rate of decolorization of the ceric ion is rapid enough to take reliable readings over a twelve-minute period. The increase in optical density, which is the measurement of the ceric-arsenite redox, ranges from 0.2 to 0.7 during this interval depending upon the sample.

Since in the Army Overseas Laboratories it is not always possible to get good reagents, a short study was made in one of these laboratories using the reagents available. These were either not chemically pure or opened with no knowledge of what had happened to them previously. The muffle furnace* used for the ashing procedure was used for all types of substances; tap water passed through an ion-exchange resin† once was used.

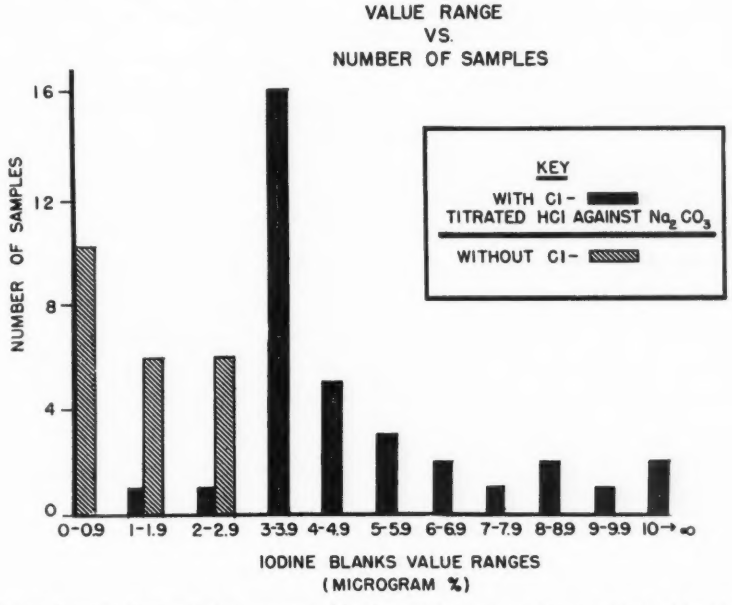

Fig. 1.—Comparison of the reagent blanks with and without added chloride ion.

PROCEDURE AND RESULTS

Because the hydrochloric acid is kept out of the procedure, it is necessary to increase the sulfuric acid concentration until it is equivalent to the total of the hydrochloric acid and sulfuric acids in the original procedures. In our laboratory the ashed sample is dissolved in 3.6 ml. of 7N sulfuric acid plus 5.4 ml. of water, instead of 3 ml. of 7N sulfuric acid, 2 ml. of 2N hydrochloric acid, and 4 ml. of water. Results are shown in Fig. 1.

It is clear that of the 22 reagent blanks which were determined without use of Cl ion as either hydrochloric acid or sodium chloride, 16 (73 per cent) were less than 2 μg per cent, and only 1 of the 22 was over 2.8 μg per cent. The 35 reagent blanks determined, with chloride ion present, ranged from 1.8 to 10.7 μg per cent with 32 (90 per cent) having values from 3.3 to 10.7 μg per cent.

*Furnace, Laboratory, Muffle Type, Army Supply No. 5-327-500.
†Deeminizer (Aloe Scientific Division, St. Louis 12, Mo.).

In the procedure published by Barker, Humphrey, and Soley,[1] their reagent blanks have values of 0.015 to 0.017 μg iodide per tube. Multiplying those values by 7/3 (100) to convert to microgram per 100 ml., the values are 3.5 and 4.0 μg per cent, which are higher than the results given previously when no chloride ion is present.

Under the conditions reported in this paper, the reaction is sensitive and rapid enough to take the readings over a twelve-minute period as in the original paper; the reagent blanks show very little or no change in optical density during this period.

The instrument used in this work was a Coleman Junior Spectrophotometer No. 6A, and 19 by 150 mm. cuvettes were used. The wave length was 420 mμ. The ashed samples were made up to 9 ml., and 4 ml. aliquots were taken for the ceric-arsenite reaction.

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A SIMPLE MICRO METHOD FOR BLOOD CARBON MONOXIDE DETERMINATION

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INTRODUCTION

CARBON monoxide (CO) is probably the most ubiquitous as well as the oldest of poisons. Its prevalence makes it immediately suspect whenever exposure to unknown toxic gases is indicated. Although air sampling is useful, it has long been recognized that the extent of exposure can best be evaluated by determining the CO content of the blood. The classic gasometric procedures developed a generation ago[1, 2] are still the most accurate and desirable when laboratory facilities and trained technicians are available. Scholander and Roughton[3] developed an ingenious microgasometric procedure, utilizing readily portable equipment, applicable to small quantities of blood. Here, too, however, considerable technical skill is required. Simpler methods have proved too inaccurate for general usage. Perhaps the most widely used of such methods is the pyrotannic acid procedure of Sayers and Yant.[4] Sendroy,[5] after critically examining this technique, concluded that the true blood CO saturation could not be determined more accurately than ± 20 per cent. With untrained personnel even greater variations might be expected. Gettler and Freemuth[6] reported a promising method of relative simplicity and rapidity, but it required 2 ml. of blood.

During World War II, Shepherd and co-workers[7] at the National Bureau of Standards developed indicating tubes for detection of minute quantities of CO in air. The remarkable specificity and sensitivity of these tubes suggested that they might be adapted to the rapid determination of CO in small quantities of blood. This report presents such a method, one in which finger blood can be analyzed by untrained personnel in the absence of laboratory facilities.

Principle.—The principle involved is similar to that of the gasometric procedures. The CO is liberated from carboxyhemoglobin by potassium ferricyanide. This liberated CO is slowly drawn through the indicator tubes, reaching into the yellow sensitized zone and causing a color change to green or blue, depending upon the quantity of CO liberated.

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MATERIALS AND METHODS

Apparatus required.—The entire assembly is illustrated in Fig. 1. It consists of:

A*	–	1	test tube (3″ by ½″)
B	–	1	2 hole rubber stopper to fit (OO)
C	–	1	straight 6″ piece of glass tubing (4 mm. OD)
D	–	1	right-angle bent piece of glass tubing (4 mm. OD)
E	–	2	short pieces of rubber tubing
F	–		National Bureau of Standards CO-sensitive tubes†
G	–	1	valve to adjust flow
H	–	1	rubber bulb (60 ml. capacity)
I	–	1	color-standard board
J	–	1	0.2 ml. pipette
K	–		cutaway view of board
L	–		cutaway view of valve.

Reagents Required.—Ferricyanide-saponin tablets–10 Gm. of potassium ferricyanide are ground and thoroughly mixed with 1.25 Gm. of saponin and a few drops of gelatin. This is sufficient to prepare about 100 small tablets containing 70 to 100 mg. each (50 CO determinations).

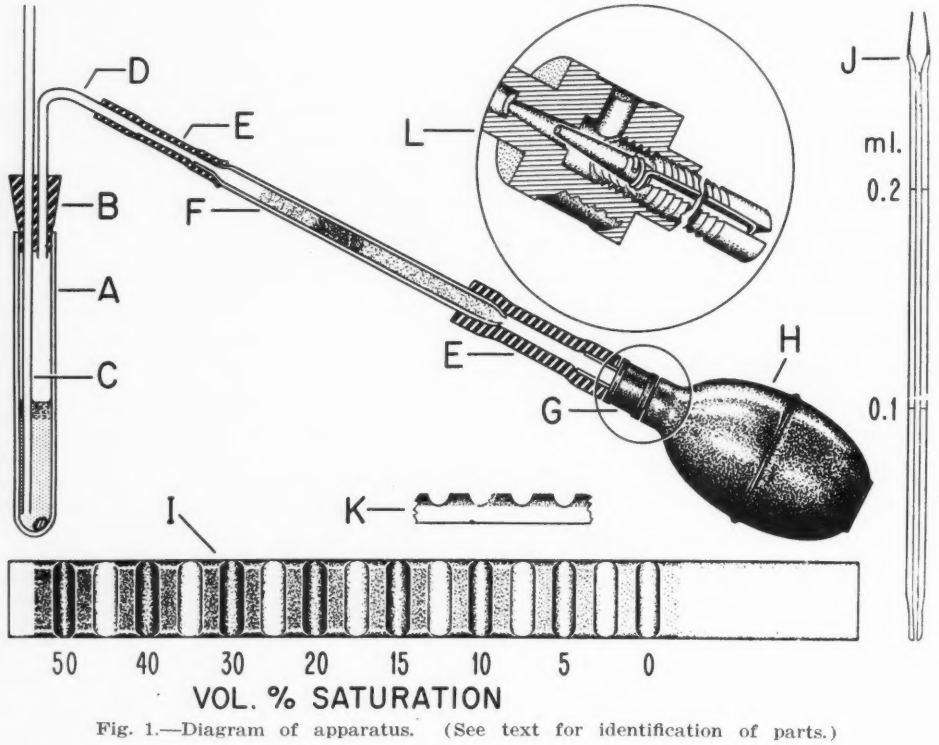

Fig. 1.—Diagram of apparatus. (See text for identification of parts.)

PROCEDURE

Into the test tube containing approximately 5 ml. of water, add a small drop of caprylic alcohol. Break the end of an indicator tube and attach the broken end to the gassing apparatus. And 0.2 ml. of the blood to be analyzed and rinse pipette into the test tube. Shake well. Add 2 ferricyanide-saponin tablets and immediately stopper test tube with gassing apparatus. Let stand, shaking occasionally, for two minutes or until the pills are dissolved.

*Letters refer to parts of diagram (Fig. 1).

†These tubes can be purchased from the United States Safety Service Company, Kansas City, and the Mine Safety Appliances Company, Pittsburgh, Pa.

Break the other end of indicator tube. Immediately evacuate bulb and attach to tube. The optimum flow rate is 90 ml. per minute.[7] If necessary, adjust valve to alter filling time. When bulb is filled, detach indicator tube, let stand thirty seconds, and compare with color standards.

Ferricyanide-saponin tablets weighing less than 100 mg. were found to disintegrate within two minutes, whereas tablets weighing 150 mg. required over three and one-half minutes. Although a single small tablet should be adequate to liberate CO from blood, we have used 2 tablets to ensure an excess.

The most accurate standards are indicator tubes which have been exposed to the CO from the indicated volume of blood with a known percentage of CO saturation. Since these colors are not stable indefinitely, periodic preparation of new standards becomes necessary. In our hands the color chart provided with the indicator tubes has not been satisfactory. Further, this color chart expresses the percentage of CO in air for the given volume sampled. The color-standard board (oil colors on a white background) shown in Fig. 1 has given us more reliable results than the charts but is less accurate than standard tubes. The white background and tube depressions facilitate comparison and the oil colors provide a permanent and reproducible standard.

RESULTS

A portion of pooled, normal, heparinized blood containing approximately 15.0 Gm. of hemoglobin per 100 ml. was saturated with CO gas. This saturated blood was then diluted with the untreated, pooled blood to produce CO saturations of 5, 10, 20, 30, 40, and 50 per cent. Aliquot portions of each sample were analyzed manometrically and by the previously described aeration method. The results are given in Table I.

It can be seen that the results with the aeration method compare favorably with the classical manometric procedure. All individual values agreed within

TABLE I. COMPARISON OF MANOMETRIC AND AERATION METHODS

BLOOD	PER CENT CO SATURATION		DIFFERENCE
	MANOMETRIC	AERATION (AVERAGE)	
1	3.0	5	+2.0
2	5.3	6	+0.7
3	7.7	13.5	+5.8
4	9.0	10	+1.0
5	9.2	11	+1.8
6	9.5	11	+1.5
7	10.8	8.5	−2.3
8	13.0	16	+3.0
9	13.2	15	+1.8
10	14.3	15	+0.7
11	14.7	15	+0.3
12	15.5	12.5	−3.0
13	16.8	22.5	+5.7
14	18.1	15	−3.1
15	18.9	20	+1.1
16	20.6	25	+4.4
17	23.9	22.5	−1.4
18	25.9	22.5	−3.4
19	30.8	30	−0.8
20	31.5	30	−1.5
21	37.9	40	+2.1
22	39.2	37.2	−2.0
23	44.6	37.2	−7.4
24	50.4	55	+4.6
25	54.1	50	−4.1
Average	21.5	22.2	+0.7

± 10 per cent saturation and four-fifths of the comparisons within ± 5 per cent saturation. The major discrepancy was with the most highly saturated samples. When these samples were diluted by using 0.1 ml. rather than 0.2 ml. of blood, the results compared closely with the manometric values.

However, it was not the objective of this study to develop a precise laboratory procedure for skilled technicians. Rather, it was hoped that approximate CO saturations could be obtained by untrained personnel. To determine the utility of this procedure in such hands, 48 Air Force basic trainees were arbitrarily selected. The educational background of this group ranged from seventh grade education to two years of college training. Approximately two-thirds (31) of the individuals had no chemistry experience whatsoever and the remainder, with one or two exceptions, had received no scientific training beyond a high school chemistry course. Directions for analysis were distributed, the procedure was demonstrated, and blood samples containing 12 to 35 per cent CO-hemoglobin were distributed for analysis. Table II summarizes the results.

TABLE II. RESULTS OF ANALYSES OF BLOOD SAMPLES FOR CARBON MONOXIDE (ANALYSES CONDUCTED BY UNTRAINED PERSONNEL)

ACTUAL CARBOXY-HEMOGLOBIN SATURATION (%)	VARIATION FROM CORRECT SATURATION			
	±5%	±5-10%	±10-20%	20%
10	34	0	1	0
12	23	6	0	0
18	44	25	5	1
35	60	0	20	0
Total	161	31	26	1

Of 219 analyses performed by completely untrained personnel, 161 (73.5 per cent) were within 5 per cent of the correct value and 87.9 per cent were within 10 per cent. Only 1 determination was in error by more than 20 per cent saturation. When it is remembered that most of these men were pipetting and matching colors for the first time, the results are highly gratifying. There was a greater tendency to overestimate than to underestimate. Thus, 54 samples were read too high and 22 too low. This tendency minimizes the possibility that a CO poison case will be overlooked. Most errors occurred at the highest saturation level (35 per cent). This can probably be attributed to the less marked color changes in this range.

DISCUSSION

After the study described here had been completed, a somewhat similar technique was reported by Waggoner and Pernell.[8] Their procedure requires 1 ml. of blood and the use of a 300 c.c. gas-sampling flask.

One difficulty occasionally encountered in the method described herein, especially with strongly CO-saturated bloods, is a "zoning" or lack of color uniformity in the indicator tubes. The lower portion, which is first exposed to the entering CO, may become considerably darker than the rest of the sensitive area. We have found that covering the lighter half with one's thumb and comparing the darker area with the standard gives more reliable results.

The use of a rubber bulb with valve adjustment is convenient, but not essential. A large (50 or 100 c.c.) syringe can be substituted satisfactorily. One must be careful to draw the gas into the syringe smoothly and slowly.

The sharpest color change occurs between 0 and 20 per cent saturation. In this range the transition from yellow to dark green is easy to match. At higher concentration levels, a bluish shade develops which does not change markedly even with rather large increases of CO. The accuracy of this technique is increased, therefore, by decreasing the blood volume of highly saturated samples and correcting accordingly. Generally, samples containing more than 20 per cent CO-hemoglobin are reanalyzed with 0.1 ml. of blood, rather than 0.2 ml., and the observed values doubled.

The exposed indicator tubes gradually darken and, therefore, must be read as soon as possible after color development. The colors may be preserved for several days by sealing the ends with paraffin. Permanent standards may be prepared if the ends of the exposed tubes are carefully sealed with a fine, hot flame.

To determine CO saturation accurately, it is necessary to know the CO capacity of the blood or the hemoglobin content. The method here described is based on a normal, young, adult male content (15 to 16 Gm. per 100 ml.). If markedly anemic or polycythemic blood is analyzed, a correction factor must be introduced. Thus, an apparent saturation of 10 per cent CO-hemoglobin would actually be 20 per cent if the hemoglobin were halved.

SUMMARY

A rapid, simple method for estimating the CO content of blood is described, whereby the CO is liberated from the carboxyhemoglobin by one reagent and determined colorimetrically by drawing the CO through another reagent in a commercially available sampling tube.

We are grateful to M/Sgt. Adam R. Resendez who performed the Van Slyke manometric analysis and to Mr. Frederick F. Jasmer and S/Sgt. Arthur Van Dyne for their aid in preparation of the color-standard board.

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METHODS FOR THE DETERMINATION OF MUSCLE CHLORIDE

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IN A study of muscle composition it became desirable to determine the chloride content of certain muscle samples both by wet-ash and dry-ash procedures. The usual methods for analysis of muscle for chloride [1a-b, 2, 3] involve an open Carius digestion with $AgNO_3$ and HNO_3, followed by a titration of the excess silver ion. An alkaline dry-ash procedure is standard for the determination of chloride in meat extracts,[1c] although this procedure has not been generally proposed for muscle samples.

In the determination of muscle chloride it is a common experience to find that slight modifications of an analytic procedure may result in 10 to 40 per cent changes in the results.[4-8] It seemed necessary, therefore, to explore the conditions under which a dry-ash procedure can be expected to yield results in agreement with those obtained by the more usual wet-ashing methods.

MATERIALS AND METHODS

About 500 grams of rat skeletal muscle was dried, defatted, and ground in a Wiley mill to pass a 40-mesh screen, and was thoroughly mixed to provide a homogeneous sample. The chloride content of aliquots of this sample was determined by four methods which are specified in Table I as alkaline ash, open Carius (A),[3] open Carius (B),[2] and water extraction.[4]

Table I. Comparison of Results Obtained From Four Chloride Methods

Method	Number of determinations	Meq. Cl^- per kilogram dry muscle	
		Mean	σ
Alkaline ash	16	71.0	1.1
Open carius (A)	8	70.1	0.8
Open carius (B)	8	72.3	0.6
Water extraction	8	70.8	1.3

In the alkaline dry-ash procedure 60 mg. of muscle sample was placed in a 50 ml. Vicor crucible with a known amount of alkali contained in 10 ml. of distilled water. The kind and amount of alkali were the experimental variables (Fig. 1). The water was slowly evaporated on a steam bath. The residue was charred with an open flame and ashed in a muffle furnace for six hours at 500° C. The ash was dissolved in distilled water, an excess of $AgNO_3$ and HNO_3 added, and the chloride content determined by a Volhard titration of the excess silver ion.[9]

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In the open Carius procedure (A), 60 mg. of muscle sample was placed in a 25 ml. Erlenmeyer flask together with 100 μl of N/10 $AgNO_3$ and 1 ml. of 6 N HNO_3. The flask was covered with a watch glass and boiled on a steam plate (hollow metal box supplied with 10 to 15 pounds of steam pressure so as to provide an operating temperature of 110 to 120° C.) for forty-five to sixty minutes. One hundred microliters of saturated ferricyanide solution was added, and the excess silver was titrated with N/10 KCNS using a 0.1 ml. Gilmont microburet. Results designated as open Carius (B) were obtained by the same procedure, with the single exception that the sample and $AgNO_3$ were added to the flask and allowed to stand together overnight[2] before the nitric acid was added. The digestion procedure and all subsequent steps were identical beyond this point.

In the water-extraction procedure, 120 mg. of muscle sample and 0.6 ml. of distilled water were sealed together in a 10 mm. Pyrex tube about 60 mm. long. The tube was heated to 85° C. for four hours, centrifuged, cooled, and opened. A 0.2 ml. aliquot of supernatant was used for the determination of chloride by a Volhard titration.

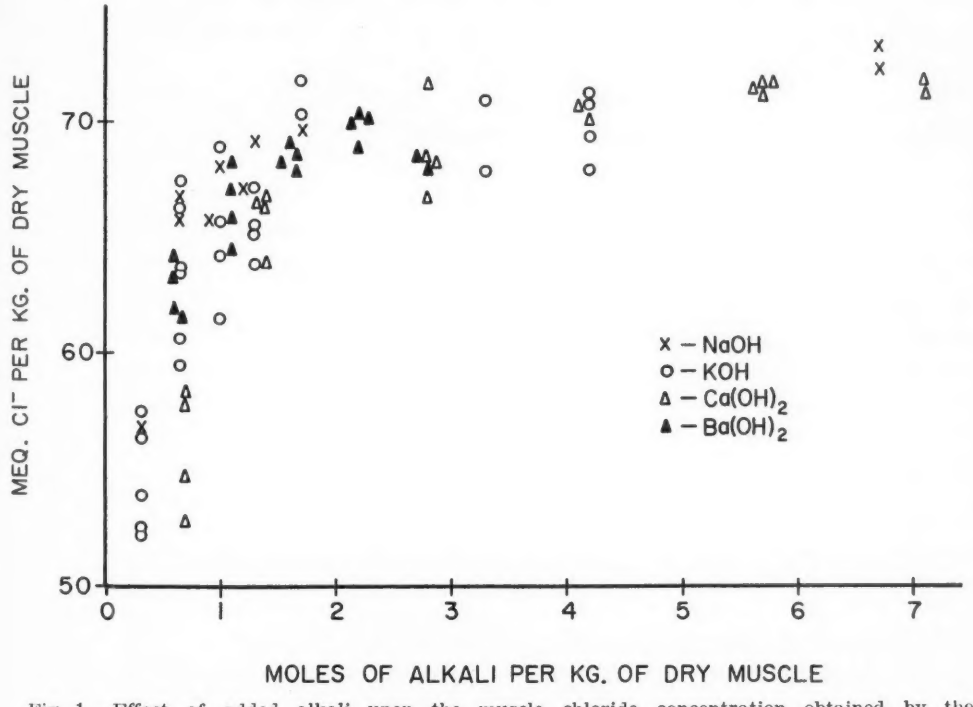

Fig. 1.—Effect of added alkali upon the muscle chloride concentration obtained by the alkaline dry-ash procedure.

RESULTS

The results obtained by the dry-ash procedure were found to be highly dependent upon the amount of alkali added to the sample prior to ashing. At low concentrations of alkali, the chloride values increased with increasing amounts of alkali until, above 2 to 3 moles per kilogram of muscle, a constant result was obtained. This result is shown in Fig. 1. The results obtained when NaOH, KOH, $Ca(OH)_2$ and $Ba(OH)_2$ were used as alkalis were substantially identical when compared on an equimolar basis. At the highest concentrations of alkali shown in Fig. 1, the ash was frequently gray instead of white. This made it more difficult to observe the end point of the Volhard titration.

Duplicate samples prepared by the same procedure were used to check the pH of the sample both before and after ashing with alkali. Even at the lowest concentrations of alkali shown in Fig. 1, the measured pH was greater than 9.5 both before and after ashing. The chloride results obtained under these conditions, however, were low by as much as 20 to 30 per cent. At alkali amounts in excess of 3 moles of alkali per kilogram of dry muscle, the determined chloride in the sample was quite constant at an average concentration of 71.0 meq. per kilogram of muscle. In Table I this result is compared with the results obtained by the three other chloride procedures which were applied to the same muscle sample. Both the mean and standard deviation are given in each case. The results obtained by the three latter methods agreed with the mean of the alkaline dry-ash data within 0.3 to 1.8 per cent, which is the same order of variation observed within any one of the methods. As a check upon the titration step, two larger aliquots were digested by open Carius method (A) and their chloride content determined gravimetrically. The mean, 71.2 meq. per kilogram, obtained in this way was, again, in agreement with the alkaline dry-ash result.

DISCUSSION

The open Carius method in its various modifications [1a-b, 2, 3, 8, 10] forms the basis of most of the commonly used methods for muscle chloride analysis. It has been stated[4-7] that if a muscle sample is wetted or extracted with water or a neutral aqueous solution as the first step in chloride analysis, a result is obtained which is 10 to 40 per cent higher than that obtained by open Carius (A). As seen in Table I, we have been unable to confirm this observation. Likewise, the increase[8] in chloride result obtained by the addition of silver nitrate prior to digestion with HNO_3 was trivial.

SUMMARY

Defatted and dried muscle samples can be satisfactorily analyzed for chloride by any of the four procedures used. Of these, the open Carius method (A) is probably the simplest and easiest to carry out. The alkaline dry-ash procedure is satisfactory, provided the samples are carefully wetted with a sufficiently large excess of alkali prior to ashing. The simple demonstration that the final ash is strongly alkaline does not constitute an adequate proof that sufficient alkali has been employed.

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OBSERVATIONS ON THE METHOD OF PRODUCING PERINEPHRIC EXPERIMENTAL HYPERTENSION

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A METHOD of producing arterial hypertension in dogs and rats that depends upon formation of an inelastic, fibroblastic hull around the kidney as a result of application of Cellophane or silk was described by Page.[1] During the past sixteen years, different types of these materials have been tried and found to vary greatly in their ability to elicit tissue reaction and formation of a perinephric hull.

Since the method is widely used, it seems desirable to report on a type of Cellophane superior to others tried. Additionally, a few salient points of the technique for wrapping the kidney, which contribute to the effectiveness of the method, are described.

METHODS

Through an incision in the left flank the transverse and longitudinal muscles are split by blunt dissection and the peritoneum opened. The left kidney is freed from its bed and delivered outside by a sweep of the fingers. Adherent fat and connective tissue is pulled away; bleeding points are easily controlled by gentle compression. A sheet of Cellophane (8½ by 11 inches) is then placed over the kidney and secured by a string tied snugly, but not tightly, around the hilus. The Cellophane is made to adhere tightly to the kidney by pulling gently at the edges below the tie. The cuff of Cellophane below the tie is reflected back over the kidney. The wrapped kidney is then dropped back into its normal position and tissue layers closed individually with interrupted silk sutures. The right kidney is removed two weeks later. Both operations are aseptic.

Cellophane is placed in Zephiran chloride solution 1:1,000 for one minute just before use and then rinsed in sterile saline. This short exposure of Cellophane to Zephiran solution is admittedly inadequate sterilization; however, long treatment with Zephiran might alter the Cellophane's effectiveness. But with this inadequate sterilization, abscess formation or peritonitis has been rare when depot penicillin was given prophylactically at the time of operation.

RESULTS

Effect of Wrapping With Cellophane #300 PT 62.—Du Pont Cellophane #300 PT 62* has proved, over a period of years, the most effective material for producing chronic experimental renal hypertension in dogs by this method.

To evaluate its effectiveness in eliciting a perinephric hull and hypertension, repeated arterial pressure determinations were done on 42 dogs after wrapping one kidney and removing the other two weeks later. Pressures were recorded with a mercury manometer after needle puncture of a femoral artery.

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Six of the 42 dogs died within one week after the second operation, the causes of death being acute infection or error in technique. Effects on arterial pressure of the remaining 36 dogs are summarized in Table I. All but 5 developed mean arterial pressures of 200 mm. Hg or more, 22 within 10 weeks, and the other 9 within 20 weeks. Approximately 90 per cent of the dogs that developed hypertension exhibited the chronic benign form; the others developed the malignant syndrome.

TABLE I. LEFT KIDNEY WRAPPED WITH DU PONT CELLOPHANE #300 PT 62; RIGHT KIDNEY REMOVED 2 WEEKS LATER

Dogs studied	36	
Hypertension developed in	31	(86%)
Hypertension developed within	1 to 2 weeks	3 dogs
	3 4	6
	5 6	4
	7 8	5
	9 10	4
Hypertension developed within	1 to 10 weeks in	22 dogs
Hypertension developed within	11 to 12 weeks	2 dogs
	13 14	1
	15 16	2
	17 18	2
	19 20	2
Hypertension developed within	11 to 20 weeks in	9 dogs

Effect of Wrapping With Other Materials.—A variety of other Cellophanes were tried; many were inactive; some produced hypertension only occasionally.

Different silks also varied greatly in their ability to stimulate formation of a perinephric hull. Some were effective in the beginning but, with time, and especially after repeated sterilization, became ineffective. The best was found to be one with the following specifications: tram silk warp and filling 10 lb. per 100 yards—1.6 oz. per square yard; weave is a 2 and 1 twill; warp 150 ends, 20/22 denier double; filling 84 picks 4th[d] 20/22 denier; porosity almost 140 cu. ft. per minute.

Since some evidence[2] points to dicetyl phosphate as being the agent in Cellophane responsible for its tissue irritating quality, different silks were treated with a suspension of dicetyl phosphate in tincture of benzoin prior to their application to the kidney. This treatment failed to improve greatly on the results obtained from application of untreated silks.

Cotton, oiled silk, nylon, rayon, and rubber sheeting were relatively ineffective.

Various plastic sprays and pastes all had too long a drying time when sprayed or painted on the kidney to make them convenient for routine use.

Snug-fitting rubber capsules, made by dipping kidney-shaped molds in liquid Vultex* and then heat drying, did not produce hypertension as consistently as did Du Pont Cellophane #300 PT 62.

*GL-15-C General Latex & Chemical Corp., Cambridge, Mass.

SUMMARY

Du Pont Cellophane #300 PT 62 is, in our experience, the most effective material yet tried for producing chronic experimental renal hypertension in dogs. It gave better results than a variety of other Cellophanes, silks, cotton, oiled silk, rayon, rubber sheeting, plastic sprays, pastes or rubber capsules. A few salient points of the technique for wrapping the kidney with Cellophane are described.

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DETERMINATION OF CARBON^{14}-LABELED ACETATE UTILIZATION BY TUBERCLE BACILLI

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METHODS for investigating the viability and state of metabolism of the tubercle bacillus are, in general, cumbersome, tedious, and often inadequate, depending as they do upon the evaluation of the rate of growth and oxygen uptake. It was felt that since acetate is assumed to be a basic unit in metabolism, the investigation of its utilization by tubercle bacilli might yield important information. Chiamori and associates[1] have reported utilization by tubercle bacilli (strain H37Rv) of acetate containing C^{14} in the carboxyl position. Long and associates[2] have studied the utilization by tubercle bacilli of methyl and carboxyl-labeled C^{13}-acetate, and have reported that part of the C^{13} is taken up and incorporated in several fractions of the bacilli and part is converted to $C^{13}O_2$ and released. A simple effective method of studying the acetate utilization by tubercle bacilli is presented here. By this method the amount of C^{14} taken up by the bacilli growing in medium containing C^{14}-acetate* is determined and correlated with the amount of C^{14} converted to $C^{14}O_2$.

MATERIAL AND METHOD

Avirulent tubercle bacilli (strain R_1R_v) were grown in Dubos' liquid Tween† albumin medium.[3] Their growth rate was measured turbidimetrically and expressed in nephelometer units (N.U.).[4]

The C^{14} uptake and the conversion of C^{14} acetate to $C^{14}O_2$ by these bacilli was measured in Conway dishes[5] (Fig. 1). Four milliliters of a suspension of tubercle bacilli, ranging in density from 30 to 1,000 N.U. per milliliter of the strongly buffered medium, was injected into the outer well of the unit, and methyl-labeled C^{14} acetate was added simultaneously.

The center well contained 1 ml. of 1 N NaOH which absorbed the liberated $C^{14}O_2$. The ground glass cover of the unit was sealed to the dish with a paraffin-petrolatum mixture. At any time samples could be removed from either well through the rubber diaphragms without danger of contamination. To facilitate comparison and conclusions, all data in this paper refer to utilization, that is, uptake and conversion, in the twenty-four-

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*The methyl-labeled C^{14} sodium acetate was obtained from Tracerlab Inc. on allocation from the Isotope Division, United States Atomic Energy Commission.

†Proprietary name for the polyoxyethylene derivative of sorbitan monooleate, obtained from Atlas Powder Co., Wilmington, Del.

hour period following addition of the acetate. Except for the one group of experiments in which the relationship of concentration of acetate to utilization was studied, an amount of acetate equivalent to 0.0189 μc was used in each dish.

At the end of the experimental period, the bacilli from the outer well were killed with 10 per cent Formalin and separated from the medium by repeated centrifugation and washing in 0.2 per cent Tween-water, in a known amount of which they were finally suspended. After determination of the number of bacilli (in N.U.), 1 ml. aliquots were plated on copper planchettes, and their radioactivity measured in a windowless continuous gas flow counter under helium as described previously for experiments on the uptake of C^{14}-labeled isoniazid by tubercle bacilli.[6] Correction for self-absorption was made[4] and the amount of C^{14} incorporated by the bacilli was expressed in disintegrations per minute (DPM) per N.U.

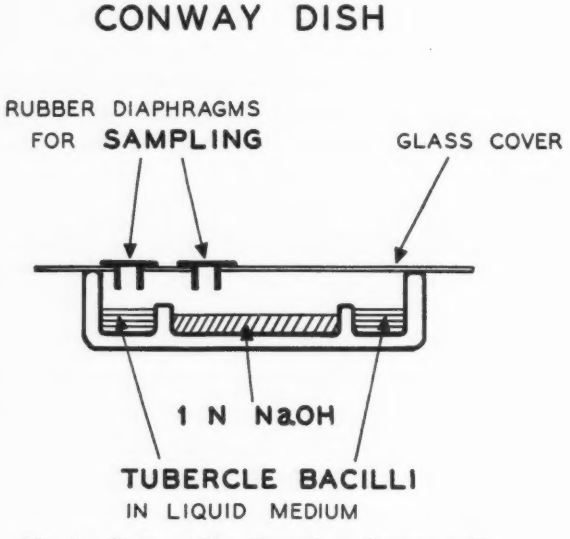

Fig. 1.—Cross section through a Conway unit.

The $C^{14}O_2$ absorbed by NaOH was precipitated as $BaCO_3$, washed, suspended in ethanol, plated on planchettes, and counted in an identical manner. Results were expressed in DPM produced per N.U. of bacilli.

In many instances the acetate utilization was compared with the tri-phenyl-tetrazolium chloride (TTC) reduction by tubercle bacilli in twenty-four hours. This determination was made as previously described[7] in test tubes with tubercle bacilli suspensions from the same cultures as those used for the acetate utilization studies.

Each individual result given represents the average of at least six determinations.

RESULTS

There was no conversion from C^{14} acetate to $C^{14}O_2$ either in Dubos' medium alone or in the cell-free filtrate of medium in which bacilli had been growing. Suspensions of Formalin-killed bacilli likewise did not utilize C^{14} acetate (Table I).

The addition of varying amounts of C^{14} acetate to suspensions of bacilli of identical age and density resulted in a C^{14} uptake and conversion to $C^{14}O_2$ which bore a direct linear relationship to the concentration of the labeled acetate added (Fig. 2).

Tubercle bacilli suspended in fresh liquid medium do not multiply for a period of time, the length of which depends upon the size of the inoculum. During this "lag-period," tubercle bacilli utilized C^{14} acetate, but did not reduce TTC. After the "lag-period," bacilli multiply at a logarithmic rate which is followed by arithmetic multiplication. It was observed that during the logarithmic phase of growth the bacilli took up considerably more C^{14} and converted slightly more C^{14} acetate to $C^{14}O_2$ than in the arithmetic phase. TTC reduction was the same in both phases. Even after ninety days in the same medium, when growth had ceased for a considerable length of time, both acetate utilization and TTC reduction were still present in appreciable amounts (Fig. 3).

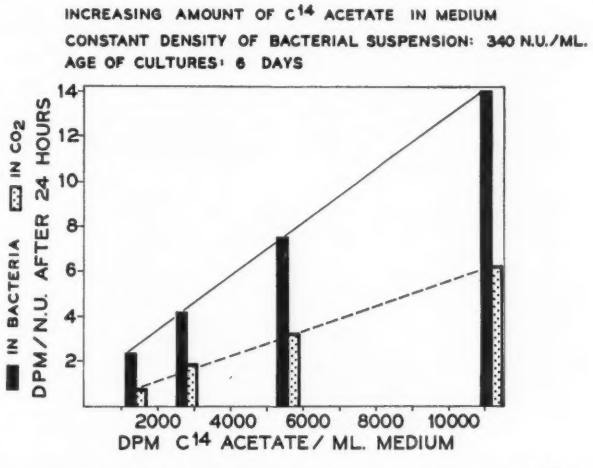

Fig. 2.—C^{14} acetate utilization by tubercle bacilli as a function of C^{14} acetate concentration in the medium.

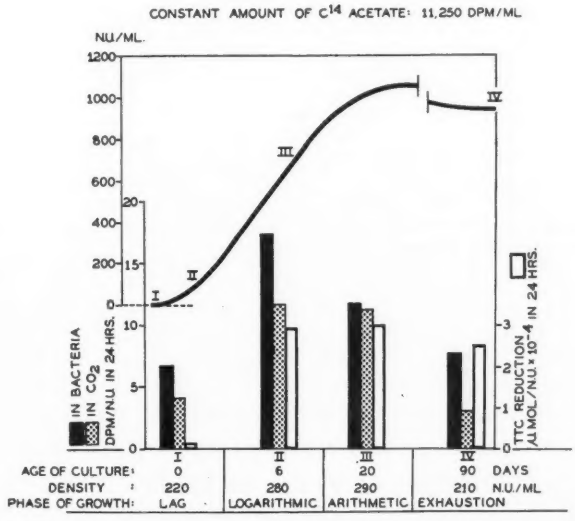

Fig. 3.—C^{14} acetate utilization and tetrazolium reduction by tubercle bacilli in different growth phases.

To demonstrate that C^{14} acetate uptake and conversion really measure some metabolic activity of the bacilli, these were submitted to varying temperatures before and during the utilization period. After exposure to 4° C. the bacilli took up and converted only very small amounts of acetate, if allowed to remain at that low temperature. Under these conditions they did not reduce TTC. If they were exposed to 4° C. for three days and then incubated at 37° C. with C^{14} acetate, they exhibited significant acetate uptake and conversion to CO_2, even though they still did not reduce TTC. Bacilli which were exposed to temperatures higher than 55° C. for thirty minutes prior to the utilization period showed very little or no acetate utilization or TTC reduction (Fig. 4).

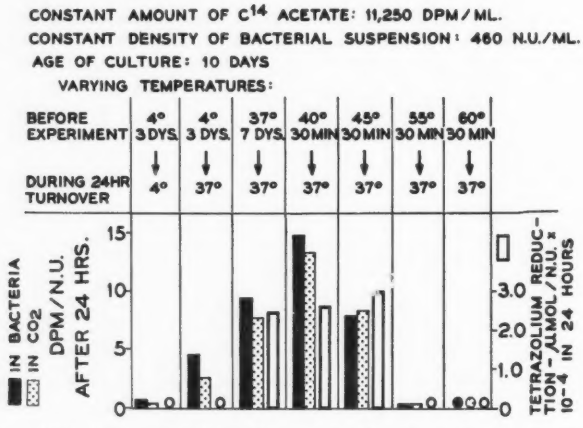

Fig. 4.—C^{14} acetate utilization and tetrazolium reduction by tubercle bacilli after and during exposure to different temperatures.

TABLE I. CONVERSION OF C^{14} ACETATE TO $C^{14}O_2$ IN LIQUID MEDIA IN THE PRESENCE OR ABSENCE OF VIABLE BACILLI

	$C^{14}O_2$ PRODUCED TWENTY-FOUR HOURS AFTER ADDING 11,250 DPM PER MILLILITER OF MEDIUM (DPM/ML.)
Dubos' medium	2
Dubos' medium with 250 N.U. viable bacilli per milliliter	1,736
Dubos' medium with 250 N.U. Formalin-killed bacilli per milliliter	0.5
Cell-free filtrate from growing cultures	3.5

During the lag-phase of growth, denser cultures took up more C^{14} and produced more $C^{14}O_2$ per N.U. of bacilli than the less dense ones (Fig. 5). After this period the reverse was true. As the density of cultures of identical age was arbitrarily increased, decreasing amounts of C^{14} were taken up by the bacilli and less C^{14} acetate was converted to $C^{14}O_2$. In parallel experiments it was found that TTC reduction was not diminished with increasing bacterial density (Fig. 6). In view of this finding, comparisons of acetate utilizations could be made only between suspensions of identical density.

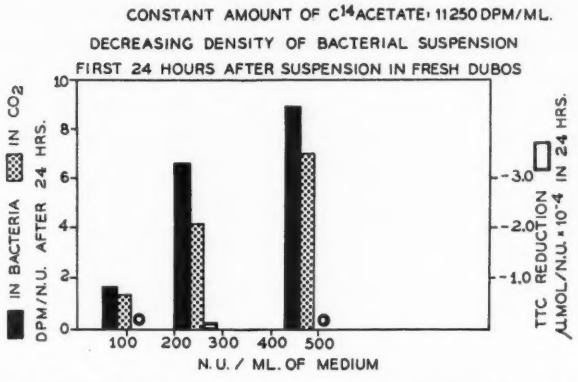
CONSTANT AMOUNT OF C14 ACETATE: 11250 DPM/ML.
DECREASING DENSITY OF BACTERIAL SUSPENSION
FIRST 24 HOURS AFTER SUSPENSION IN FRESH DUBOS
IN BACTERIA IN CO2
DPM/N.U. AFTER 24 HRS.
TTC REDUCTION
µMOL/N.U. × 10-4 IN 24 HRS.
N.U. / ML. OF MEDIUM

Fig. 5.—C14 acetate utilization and tetrazolium reduction by tubercle bacilli in the "lag-phase" of growth as a function of bacterial density.

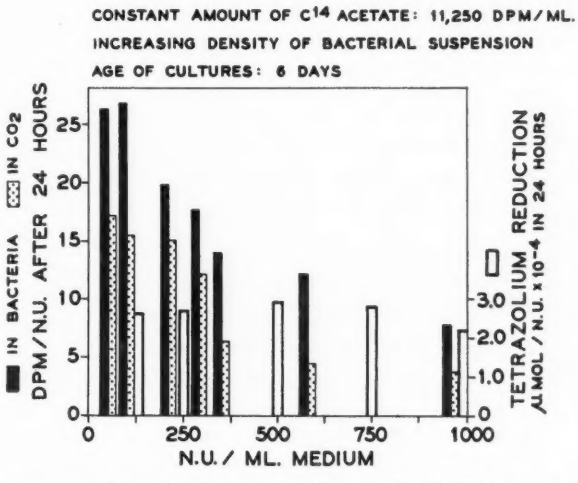
CONSTANT AMOUNT OF C14 ACETATE: 11,250 DPM/ML.
INCREASING DENSITY OF BACTERIAL SUSPENSION
AGE OF CULTURES: 6 DAYS
IN BACTERIA IN CO2
DPM/N.U. AFTER 24 HOURS
TETRAZOLIUM REDUCTION
µMOL/N.U. × 10-4 IN 24 HOURS
N.U. / ML. MEDIUM

Fig. 6.—C14 acetate utilization and tetrazolium reduction by tubercle bacilli in the logarithmic phase of growth as a function of bacterial density.

DISCUSSION

Although it has been reported that human tubercle bacilli will not grow in a synthetic medium with acetate as the only carbon source,[8] it has been shown by the use of carbon isotopes that acetate is utilized in more complex media.[2] Acetate is one of the fundamental units in metabolism and therefore it could be expected that its utilization by tubercle bacilli represents basic metabolic activity.

The fact that acetate is utilized only in the presence of living tubercle bacilli (Table I) indicates that this represents metabolic activity and not chemical reaction with a component of the medium nor with any substance secreted into the medium by the bacilli.

There was increased acetate utilization during the logarithmic phase of growth as compared with the arithmetic phase. It has been shown that the duration of logarithmic growth of tubercle bacilli depends upon the oxygen

available in the medium,[9] and it can be assumed that acetate utilization also is dependent upon the presence of oxygen and for that reason is greater in the logarithmic phase. This hypothesis is strengthened by the fact that TTC is equally reduced in the logarithmic and arithmetic phases of growth, for the presence of oxygen is not necessary for TTC reduction since it is reduced anaerobically as well as aerobically.[10]

The fact that acetate utilization and TTC reduction is still considerable several weeks after cessation of growth indicates continued metabolic activity.

It was felt that there is probably some significance in the ratio between the C^{14} incorporated into the cell and the $C^{14}O_2$ produced under given conditions. Bacilli damaged by exposure to 45° C. show a decrease in the ratio between the C^{14} incorporated and the $C^{14}O_2$ produced. Work now in progress indicates that tubercle bacilli damaged by other agents, e.g., tuberculostatic drugs, exhibit the same altered acetate utilization. This increase in $C^{14}O_2$ production as compared with a relative decrease in C^{14} incorporation may represent less synthesis of cell substance.

The diminished metabolic activity of denser suspensions of bacteria has been described as the "crowding effect.[11] Again, probably, the optimal amount of O_2 is not available to the bacilli in the denser cultures. This again seems to be substantiated by the fact that TTC reduction did not diminish with increasing bacterial density. The reversal of the "crowding effect" during the lag-phase lends weight to the theory mentioned by Hinshelwood[11] that the inverse relationship between the size of the bacterial inoculum and the length of the lag-phase is a function of the synthesis by the bacteria of some essential metabolite, which must be at an optimal concentration before cell division can occur. Similarly, greater synthesis of this metabolite by a greater number of bacilli allows more acetate utilization.

The studies with C^{13} acetate reported by Long and his associates[2] were carried out by chemical fractionation of bacteria harvested after six weeks from surface growth on media containing the methyl- or carboxyl-labeled acetate. The activity of the fractions indicated a wide distribution in all cellular components. The amounts of $C^{13}O_2$ trapped as the sodium or barium carbonate proved that there is considerable conversion of acetate to CO_2. Chiamori and his associates[1] harvested 3-week-old surface growth cultures, added carboxyl-labeled acetate, and, after 21 hours' incubation, extracted the lipid fraction. No difference in activity between the untreated and the streptomycin-treated bacilli was found.

The method described here allows a dynamic evaluation of a metabolic activity of a small number of growing bacteria at any age and offers the further advantage of allowing a quantitative determination of the relationship between incorporation of acetate into the cell substance and conversion to CO_2. Certainly the utilization of other substances could be investigated in the same manner. The ease with which samples can be taken from the outer and the

center well at any time permits the study of the progress or inhibition of the utilization. The method has wide applicability to any metabolic system which can be maintained in the Conway units.

SUMMARY

A method for study of acetate utilization by tubercle bacilli is presented. It consists of adding C^{14} methyl-labeled acetate to the microorganisms in Conway units and determining the C^{14} uptake and the conversion to $C^{14}O_2$ by the bacilli.

Study of the acetate utilization during different phases of growth and after submitting the cultures to varying temperatures indicates that the method described allows an evaluation of a metabolic activity of a small number of bacteria at any age and under different metabolic conditions.

Parallel determinations of tri-phenyl-tetrazolium chloride reduction indicate that this is independent of O_2 availability, while the acetate utilization seems to depend upon O_2 available to each microorganism.

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PROCEEDINGS OF THE CENTRAL SOCIETY FOR CLINICAL RESEARCH

Twenty-Eighth Annual Meeting

Chicago, Ill., Nov. 4 and 5, 1955

ABSTRACTS

59. THE IMMEDIATE EFFECT OF CHEWING TOBACCO ON THE CIRCULATION OF HABITUAL MALE CHEWERS

ARNOLD IGLAUER, M.D., DAVID L. SIMON, M.D. (BY INVITATION), AND ROBERT E. RAKEL (BY INVITATION), CINCINNATI, OHIO

Despite the annual consumption in the United States of 81 million pounds of chewing tobacco, practically no information is available about its clinical or physiologic effects. Accordingly, 25 habitual male chewers were studied before, during, and after chewing both standard commercial (1.53 per cent nicotine) and low nicotine (0.31 and 0.49 per cent) tobacco. Chewing gum was used as a control in some cases. In addition, 50 leading physicians interested in cardiovascular disease were circularized concerning their clinical experience with chewing tobacco.

Studies were carried out in the basal, postabsorptive state at 78° F. and 40 per cent relative humidity. Subjects were supine and unclothed, and continuous temperature readings were made by copper constantin fine-wire thermocouples attached to the forehead and the volar surfaces of fingers and toes, employing a Brown constant recording potentiometer. Blood pressures and pulse rates were determined. Ballistocardiograms were recorded with a high frequency table type research instrument.

Forehead temperatures remained constant with both types of tobacco. In most cases, the pattern of temperature changes was similar to that produced by smoking. Finger temperatures fell in most subjects, toe temperatures dropping less. Changes were usually less marked with low-nicotine tobacco, absent with chewing gum. Blood pressures and pulse rates increased in all cases with standard tobacco. The ballistocardiographic patterns deteriorated in most cases after chewing standard tobacco, remaining unchanged after chewing gum. No consistent change occurred in computed initial cardiac force (Starr, 1955). Results of the questionnaire revealed little knowledge of the clinical effect of chewing tobacco.

60. "ALARM REACTION" AND NORMAL BLOOD PICTURE IN MACACA MULATTA

MARGARET IVES, PH.D. (BY INVITATION), AND GAIL M. DACK, M.D., PH.D., CHICAGO, ILL.

A significant decrease occurred in the white blood cell count following the daily handling of the animals over a period of months. Thirty-six normal monkeys of both sexes were maintained in a laboratory colony for one year in excellent health. Body weights, hemoglobin, red and white blood cell counts, and differential white cell counts with the standard error of the mean are reported for these monkeys. All data have been tested statistically and no differences were found among individual animals, lots, or sexes which would obscure the accuracy of these figures. An explanation of the effect of the "alarm reaction" due to handling on the white blood count is also given.

61. FAMILIAL PRIMARY SYSTEMIC AMYLOIDOSIS

CHARLES E. JACKSON, M.D., JOHN G. RUKAVINA, M.D., WALTER D. BLOCK, PH.D., HAROLD F. FALLS, M.D., AND JOSHUA CAREY, M.D., ANN ARBOR, MICH., AND BLUFFTON, IND.

(INTRODUCED BY ARTHUR C. CURTIS, M.D.)

An electrophoretic pattern with an abnormal peak in the alpha$_2$ area is described with evidence suggesting its diagnostic importance in primary systemic amyloidosis. One hundred and sixty-six individuals have been studied by the free electrophoretic technique with abnormal proteins being found in 78 individuals from 5 apparently distinct families of Swiss descent. Pedigrees are presented which seem to indicate that primary systemic amyloidosis in this group is inherited as a simple Mendelian dominant condition. The clinical manifestations in these individuals were quite varied, with peripheral neuropathy as the predominant symptom along with visual disturbances due to amyloid deposits in the vitreous, and congestive heart failure in some. The heart of the propositus of this study weighed 840 grams at autopsy and had extensive amyloid deposition. Many individuals with the abnormal protein were found to be indistinguishable otherwise from normal individuals although some of these even showed marked aberrations in their lipoprotein values. This study illustrates how a genetic problem may be refined by use of some of the newer biochemical techniques.

Enclosed with each abstract are two charts showing the pedigrees of the protein abnormality in these families along with a table showing the lipoprotein values in 26 of the individuals with primary systemic amyloidosis. A reprint of an article previously published which describes the abnormal electrophoretic pattern is also enclosed.

62. CORRELATION OF HISTOPATHOLOGY AND CLINICAL STUDIES IN PYELONEPHRITIS

GEORGE GEE JACKSON, M.D., K. PETER POIRIER, M.D. (BY INVITATION), AND FREDERICK D. DALLENBACH, M.D. (BY INVITATION), CHICAGO, ILL.

The morphologic and clinical characteristics of chronic pyelonephritis have been related largely from studies based upon autopsy material and retrospective examinations of the clinical histories. The occurrence of renal

insufficiency from pyelonephritis in the absence of symptoms has been common. The development of a suitable technique for needle biopsy of the kidney has enabled correlation of the pathologic lesion of the kidney with clinical and laboratory data. We have attempted percutaneous renal biopsy 75 times upon 62 patients who had infected urine and variable symptoms suggesting pyelonephritis. Adequate tissue for histologic diagnosis and bacteriologic culture was obtained in 80 per cent of the biopsies, but 16 or 20 per cent of attempted biopsies were unsuccessful. No fatality was incurred. Minor complications occurred following 15 per cent of the procedures, and serious complications such as hemorrhage or bacteremia were encountered in 7 patients or after 9.3 per cent of the biopsies attempted. Eight patients have had biopsies of both kidneys and serial biopsies have been performed upon 4 patients.

Among 50 patients with adequate biopsies 20 per cent had no morphologic renal lesion, and 10 per cent had no lesion attributable to pyelonephritis; pyelonephritis was secondary in 14 per cent and primary in 56 per cent of these patients. Photomicrographs characteristic of the different stages of pyelonephritis will be shown.

The degree of histopathologic abnormality has been related to the nature of the clinical symptoms, and these aspects have been correlated with the bacterial etiology of the infection. There is a suggestion that gram-negative bacilli commonly produce acute symptoms without appreciable kidney damage while gram-positive cocci are more commonly associated with diffuse renal disease often with meager symptoms. Forty-five per cent of the bacteriologic cultures of the kidney tissue were positive. The results could not be predicted from the clinical symptoms but had a positive correlation with the severity of the pathologic lesion.

The measurement of renal plasma flow, glomerular filtration rate, and maximal tubular secretion of para-aminohippuric acid failed to show significant disproportion of glomerular and tubular function in patients with histologic evidence of primary pyelonephritis.

63. INTERMITTENT CLAUDICATION

CARL A. JOHNSON, M.D., CHICAGO, ILL.

The material to be presented is based upon an experimental study of four patients with arteriosclerosis obliterans with symptoms of intermittent claudication as well as rest pains in some of the patients. The systolic blood pressure was recorded in all four extremities on a recording oscillometer using the double-cuff method. The diastolic blood pressure was recorded in all four extremities with the same instrument using Marey's principle which indicates that the diastolic pressure is at the cuff pressure level at which the maximum pulse wave amplitude is recorded. The pulse volume change which is calibrated and corrected for overshoot and "run off" and expressed in milliliters per minute was recorded on the oscillometer at various levels in the extremities.

The following studies were made: (1) The immediate effects of one-half-hour massage to one leg on the corrected minute pulse volume change was recorded at both ankles; (2) the late effects of one-half-hour daily massage to the affected legs on the corrected minute pulse volume change was recorded at the ankles once a month for over one year; (3) the corrected minute pulse volume change was recorded at the ankles before, during the pain of intermittent claudication, and after ten- and twenty-minute rest periods; (4) the mechanism of the "paradoxical pulse" was studied; and (5) a clinical evaluation of massage for intermittent claudication has been made.

In summary, this study indicates that in addition to the organic arterial occlusion, the vasospastic element is extremely important in the genesis of intermittent claudication. The discussion will be illustrated by lantern slides of the method of study, the graphic recordings and tables summarizing the study.

64. SERUM VITAMIN B_{12} CONCENTRATIONS IN LIVER DISEASE

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(INTRODUCED BY RICHARD B. CAPPS, M.D.)

Serum vitamin B_{12} concentrations were studied in a group of patients with various types of liver disease. The bio-assay method utilizing the growth response of *Euglena gracilis* was used. This method measures both the free and combined vitamin B_{12} in concentrations as low as 10 $\mu\mu$g per milliliter.

The mean values obtained for the normal controls were 72 $\mu\mu$g per milliliter for free serum vitamin B_{12} and 248 $\mu\mu$g per milliliter for the combined serum vitamin B_{12}. Thirty-five patients were studied with serial determinations for over one year. Eleven patients had Laennec's cirrhosis, ten had postnecrotic cirrhosis, four had biliary cirrhosis, six had acute viral hepatitis, and four were studied during periods of hepatic coma. All the patients with the exception of those with biliary cirrhosis, who had values within the normal range, had vitamin B_{12} serum concentrations 3 to 8 times the normal values. Patients in hepatic coma had values between 30 and 40 times the normal. Patients with Laennec's cirrhosis had proportionate rises in both the free and combined vitamin B_{12} while those with acute hepatitis and those with postnecrotic cirrhosis had a greater proportional rise in the free serum vitamin B_{12}.

A limited number of determinations were made on twenty-four-hour urine collections from these patients and it was found that these patients excreted from 10 to 30 times the normal amount of vitamin B_{12} in the urine, all of which was in the free form.

It would seem fairly clear from these results that the metabolism of vitamin B_{12} is seriously altered both in acute and chronic liver disease. The theoretical implications of these results will be discussed.

65. HEMOLYTIC ANEMIA IN MYELOFIBROSIS WITH SPLENOMEGALY

DONALD R. KORST, M.D. (BY INVITATION), DALLAS V. CLATANOFF, M.D. (BY INVITATION), AND ROBERT F. SCHILLING, M.D., MADISON, WIS.

Studies in 23 patients in whom myelofibrosis was demonstrated by bone-marrow biopsy have shown that an increased rate of hemolysis is common. Hemoglobin levels ranged from 3.4 to 16 Gm. per 100 ml. Reticulocyte percentages were between 0.9 and 8.4, 16 patients having more than 3 per cent reticulocytes. Circulating normoblasts were seen in all but one patient. Fecal urobilinogen excretion exceeded 300 mg. per day in 9 of the 13 patients so studied. Erythrocyte life span, measured in 4 instances with the radiochromate technique, was definitely shortened. Plasma iron turnover rates were increased, but in 2 patients the incorporation of radio-iron into circulating erythrocytes was definitely below normal. In 2 patients there was a severe hemolytic process which was markedly alleviated following splenectomy. The direct Coombs test was negative in these 2 and the 7 other patients in whom it was made. Osmotic fragility of erythrocytes was not increased in the 12 bloods so tested.

Summary. A mild to severe hemolytic process is common in myelofibrosis with splenomegaly and may be a major cause of anemia in patients with a reduced capacity for erythropoiesis.

66. THE ROUTINE MEASUREMENT OF RESPIRATORY RATE; AN EXPENSIVE TRIBUTE TO TRADITION

Ross C. Kory, M.D., Milwaukee, Wis.

(Introduced by Maurice Hardgrove, M.D.)

The measurement and charting of the respiratory rate has been a routine procedure in our hospitals for many years. Serial determinations of respiratory rate have been quite useful in the past, particularly as a prognostic aid in lobar pneumonia. Today, however, because of modern diagnostic and therapeutic advances, the routine measurement and charting of respiratory rates is clinically useful in only a small minority of hospital patients. The medical need for respiratory rate measurements was assessed by: (1) chart review of 300 patients in two hospitals, a total of 11,175 patient days; and (2) by a survey of 40 physicians actually in charge of 1,067 patients. In both surveys the actual medical need for respiratory rate measurements was considered to be slightly less than 5 per cent of the patient days. The accuracy of routine respiratory rate determinations was assessed by comparing, in 109 patients, the respiratory rates carefully measured for this study with the rates routinely recorded on the ward. There was no correlation between the two sets of measurements, the routine rates correlating only with the figure "20." A review of the hospital charts on 248 unselected male patients in two hospitals revealed that of 13,710 respiratory rates recorded on these patients, 12,895 or 94 per cent fell in the range of 18 to 22 per minute.

Over 50 million hours of nursing time are spent annually in measuring and recording respiratory rates in the 654,000 general hospital beds in the United States. Ninety-five per cent of these measurements in all probability are of little medical value. The inclusion in hospital charts of data as inaccurate as these measurements constitutes poorer medical practice than the omission of such data.

It is concluded that more accurate and useful information would be obtained if respiratory rates were measured only where specifically ordered by the physician, and that such measurements would include an evaluation of the character of breathing as well as the rate. The substitution of such a practice for the routine now in general use would result in more useful and accurate information and at the same time effect an enormous savings in nursing time.

67. THE BLOOD PRESSURE EFFECT OF RAUWOLFIA SERPENTINE AND RESERPIN IN ESSENTIAL HYPERTENSION; A LONG-TERM DOUBLE-BLIND STUDY

J. Harold Kotte, M.D., Robert Helm, M.D., Murray B. Sheldon, M.D. (By Invitation), Isom C. Walker, M.D. (By Invitation), William Stiles, M.D. (By Invitation), and James Evans, M.D. (By Invitation),
Cincinnati, Ohio

A total of 15 patients with benign essential hypertension were treated in a conventional double-blind study over a period of 22 months, during which time they received either Rauwolfia serpentine 50 mg., 4 times daily, or Reserpin 0.25 mg., 4 times daily, or an identical placebo for alternate periods of 6

to 12 weeks. All patients were observed for a variable period of time during which they received no medication or placebo. During the entire period blood pressures were checked in the supine, seated, and standing positions. A random selection of patients were also followed independently in group psychotherapy. Blood pressure readings were subjected to statistical analysis by the method of *Analysis of Variance.* The results indicated that there was a statistically significant blood pressure effect of the drugs over the placebo and no medication, namely, the systolic blood pressure in 6 patients and the diastolic blood pressure in 8 patients. This effect was highly significant in 4 and 6 patients on the systolic and diastolic pressures, respectively. One patient had a highly significant blood pressure response to the placebo as compared with the no therapy. One patient had a significantly poor response to the drug as compared with the placebo or no therapy. There were no instances in which there appeared to be a significant postural blood pressure effect during any of the observed periods.

68. A RATIONAL THERAPY OF SYSTEMIC LUPUS ERYTHEMATOSUS

N. B. Kurnick, M.D., Long Beach, Calif.

Our studies on the mechanism of the L.E. cell phenomenon lead to the conclusion that the abnormal factor in the gamma globulin of the patient alters the cell membrane, admitting a serum proteolytic enzyme (in Fraction III) into the cell, which destroys the protein inhibitor of desoxyribonuclease (DNase). As a consequence, the enzyme is liberated, attacks the chromosomal desoxyribonucleic acid, yielding the depolymerized nuclear mass which characterizes the phenomenon.

The inhibitor of DNase, in vitro, is shown to permeate the cell wall. Its addition to the L.E. serum-normal leukocyte system inhibits the L.E. cell phenomenon. Its intramuscular injection reduces circulating serum DNase activity for 48 to 72 hours.

We have treated a number of patients (10 with follow-up of 4 months or longer) with homologous fresh whole blood intramuscularly. The lysis of the infused leukocytes provides (1) leukocyte cell walls which may absorb the abnormal circulating gamma globulin, and (2) DNase inhibitor which replaces the intracellular inhibitor destroyed in the mechanism of the L.E. cell. One patient was treated with leukocyte fractions containing DNase inhibitor but no cell particulates, cell particulates alone, and a mixture.

The results are very encouraging. Skin lesions cleared within one week in all. The serum DNase activity fell and L.E. cells disappeared usually within 3 to 4 weeks. Arthralgias, myalgias, and fever subsided within a month. Renal damage showed improvement in several patients, but one uremic patient died in uremic coma despite treatment. Steroids were successfully withdrawn in almost all cases, with temporary withdrawal symptoms in some.

69. THE SIMULTANEOUS CORRECTION OF PLAIN-WATER DEFICIT AND EXTRACELLULAR FLUID VOLUME LOSS IN NONTROPICAL SPRUE TREATED WITH PREDNISOLONE

John A. Layne, M.D., and C. R. Paynter, M.D. (By Invitation),
Great Falls, Mont.

Prolonged loss of gastrointestinal secretions results in loss of electrolyte plus water, representing extracellular fluid volume loss, and produces hypovolemia. Loss of an excess of plain-water (true dehydration) may be expected

to occur also in the patient who is losing gastrointestinal secretions, since proportionately more water than electrolyte is lost, as compared with isotonic salt solutions. The simultaneous replacement of plain-water deficit and extracellular fluid volume loss corrects the hemodynamic effects resulting from volume loss, and permits normally functioning kidneys, with minimal optimal amounts of electrolyte to work with, to correct electrolyte deficiency patterns with remarkable exactitude. These principles which have been established as valid in the treatment of conditions involving excessive loss of gastrointestinal secretions where only sufficient and proper replacement are required, have also been found to apply to conditions having defects in absorption from the gastrointestinal tract, such as nontropical sprue. The following observations were made in a 26-year-old woman, first diagnosed as celiac disease at age 3 years, who was admitted with a marked exacerbation of her steatorrhea-diarrhea. She responded promptly to the administration of 40 mg. of Prednisolone daily, her diarrhea ceasing, and her stools becoming formed within 5 days. Plasma sodium values were normal during the period of observation. The severe hypopotassemia present on admission was corrected by dietary potassium plus daily oral supplement of but 2 Gm. (27 meq.) of potassium chloride. A positive sodium balance and an even greater positive potassium balance occurred during treatment. Furthermore, the amount of water retained was greater than would be expected to occur as a result of the positive sodium balance, and without the development of edema. Prednisolone appears ideally suited for the treatment of sprue since it did not produce increased urinary excretion of potassium, nor retention of sodium. Maintenance of this patient was accomplished on much smaller doses than used during the initial period of treatment.

70. THE MECHANISM OF HEMORRHAGIC MANIFESTATIONS ASSOCIATED WITH THROMBOCYTHEMIA

WILLIAM C. LEVIN, M.D., D. R. CELANDER, PH.D. (BY INVITATION), AND MASON GUEST, PH.D. (BY INVITATION), GALVESTON, TEX.

An opportunity to study the mechanism of hemorrhagic symptoms associated with thrombocythemia has been afforded us during observation and management of an 11-year-old white girl, who had had recurrent episodes of bleeding, usually associated with trauma, during the two years prior to hospitalization. On admission, her platelet count was 5,500,000 and complete examination of the peripheral blood and bone marrow indicated the presence of a myeloproliferative disease, primarily involving megakaryocytes. Bleeding time was prolonged although the Lee and White coagulation time, clot retraction, and Quick prothrombin time were normal.

During the next week, a number of subcutaneous nodules developed, and were proved to be loculated hematomas by biopsy. Simultaneously, the platelet count dropped to 2,500,000 and the two-stage prothrombin assay revealed 20 per cent prothrombin content. Fibrinogen was also found to be moderately reduced (160 mg. per cent). There was prompt recovery of prothrombin to above 100 per cent and a gradual rise in fibrinogen to normal levels. Thereafter, the platelet count rose to levels of 5,000,000 and above. No new hemorrhagic symptoms developed. One month later, administration of radioactive phosphorus produced a reduction of platelets to about 2,500,000. With this reduction, there was no concomitant decrease in prothrombin or fibrinogen, nor was there hemorrhage.

The sequential occurrence of these phenomena suggest that the initial reduction in platelets, obviously unrelated to any marrow depression, was due to a sudden intensive lysis of platelets in the peripheral blood. It is further suggested that this liberated sufficient platelet accelerator globulin and various components of the thromboplastic system to produce rapid utilization of prothrombin to an extreme degree and of fibrinogen to a lesser degree, in the course of intravascular coagulation. Since no endothelial defects were present, no thrombi were formed. However, the extreme reduction of plasma prothrombin content could account for the development of hemorrhagic symptoms. The patient was hemorrhagic only during the brief period in which plasma prothrombin content was seriously deficient.

Although other factors may be operative, it seems that the hypothesis here presented is sufficient to account for hemorrhagic manifestations in patients with thrombocythemia.

71. A TRIAL IN DOGS OF METHODS SUITABLE FOR ESTIMATION OF MYOCARDIAL Rb^{86} UPTAKE RATE IN MAN, AND THE EFFECT OF l-NOREPINEPHRINE AND PITRESSIN ON Rb^{86} UPTAKE

W. D. Love, M.D. (By Invitation), and G. E. Burch, M.D., New Orleans, La.

Following Rb^{86} injection approximately 70 per cent of the isotope initially delivered to the heart in the coronary blood enters the myocardium. Since the rate of coronary blood flow is a factor limiting Rb^{86} uptake, methods suitable for estimating Rb^{86} uptake rate in man were tested in 27 dogs. Steady plasma radioactivity was obtained by injecting 0.5 mc. Rb^{86} at a decreasing rate and monitoring arterial blood in a small external arteriovenous shunt. Precordial radioactivity was recorded continuously by a scintillation ratemeter collimated so that approximately two-thirds was derived from myocardium. Rb^{86} and K concentrations were determined in serial samples of arterial plasma and specimens obtained from the heart at sacrifice.

Rb^{86} was considered to trace potassium, and myocardial K was treated as a homogeneous pool exchanging at a single rate. Turnover rate of myocardial K was obtained by differentiation of the precordial radioactivity curve; and myocardial Rb^{86} clearance was calculated using the empirical relationship of precordial radioactivity to myocardial Rb^{86} concentration ($r = +0.93$). These estimates were then compared with data obtained by direct analysis of the heart.

In six dogs, infusion of 0.065 pressor units Pitressin per kilogram per minute decreased apparent K turnover rate determined at sacrifice from the normal average of 2.8 per cent per minute to 1.4 per cent per minute; and Rb^{86} clearance fell from the normal 30-minute average of 50 ml. plasma per 100 Gm. ventricle per minute to 34 ml. In four dogs, infusion of 2.5 μg l-norepinephrine per kilogram per minute increased turnover rate to 5.4 per cent per minute. In three this was caused by low myocardial K, clearance remaining normal.

Rapid turnover induced by l-norepinephrine was apparent from differentiation of the time course of precordial radioactivity in all four dogs, but slow turnover caused by Pitressin was detected in only two of six. This method was unreliable, probably because of slowly exchanging skeletal muscle components and failure to obtain a stable plasma Rb^{86}/K ratio in some instances. Predictions of clearance were more reliable (standard error of estimate 10 ml.), and reduced clearance was detectable in all dogs infused with Pitressin.

The results warrant trial of these procedures in man.

72. STUDIES OF THE INHIBITORY EFFECT OF L-TRI-IODOTHYRONINE ON I^{131} UPTAKE BY THE THYROID GLAND IN EUTHYROIDISM AND EXOPHTHALMIC GOITER

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(INTRODUCED BY F. RAYMOND KEATING, JR., M.D.)

Starr and also Werner showed that euthyroid persons given large enough doses of l-tri-iodothyronine for 1 week will experience pronounced inhibition of collection of I^{131} by the thyroid. Such studies have compared one observation of I^{131} uptake before and one after administration of the hormone for 1 week.

The present study was undertaken to follow the development of thyroidal inhibition by observing the uptake of I^{131} at more frequent intervals. Thirty-eight euthyroid persons were given l-tri-iodothyronine (Smith, Kline & French Laboratories) daily for 3 to 32 days. The same dose was given each day to each person but it varied among persons from 3.5 to 140 μg per day. A small I^{131} tracer was given to each person before the daily administration of l-tri-iodothyronine was begun; after this, repeated small tracers were given, sometimes daily and sometimes at intervals of 2 or 3 days. Observations were made on the speed and degree of inhibition of I^{131} uptake produced by the various daily doses of l-tri-iodothyronine, the length of time required for the thyroid to "escape" from the inhibitory effect of l-tri-iodothyronine, and the thyroidal response to injection of thyrotropin after suppression of I^{131} uptake by l-tri-iodothyronine.

It was found that l-tri-iodothyronine in daily doses of 3.5 to 17.5 μg had a limited and variable suppressive effect on thyroidal uptake of I^{131} by normal persons. In daily doses of 25 μg or more, l-tri-iodothyronine invariably suppressed uptake; the larger the dose, the more rapid was the inhibition. At a dose of 50 μg per day, most euthyroid persons experienced 50 per cent inhibition of their original uptakes of I^{131} within 3 to 4 days. In seven patients with exophthalmic goiter, little or no inhibition was evident in periods up to 2 weeks when the dose of l-tri-iodothyronine was 50 to 140 μg per day. Administration of thyrotropin restored the ability of the inhibited thyroid gland to collect I^{131} in normal or increased amounts.

73. AN IN VIVO METHOD FOR THE DETERMINATION OF CARDIAC OUTPUT

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(INTRODUCED BY E. LEE SHRADER, M.D.)

The validity of dye dilution methods for the determination of cardiac output seems well established. Also well established is the fact that radioiodinated human serum albumin and dyes have a similar distribution during the early moments following an intravenous injection. Cardiac output values obtained with procedures using radioiodinated albumin have been shown to compare favorably with cardiac output values obtained by gas analysis methods utilizing the Fick principle. There have been published at least two reports which describe an in vivo method for caridac output determination using radioiodinated albumin. This presentation concerns studies aimed at determining the validity of the calibration technique required for such external counting methods.

In the procedure employed, a directional scintillation counter is positioned over the second left intercostal space adjacent to the sternum. Five to 15 μc of radioiodinated albumin then are injected into an antecubital vein. The rapid changes in the radioiodine concentration of the blood which follow are reflected on a curve inscribed by the graphic recorder of the in vivo counting system. Ten minutes later, with the position of the external counter unchanged, a venous blood sample is obtained for calibration. Once the calibration factor for the individual determination is known, a cardiac output value may be calculated from the standard formula for dye dilution methods.

Twenty-two normal adult men were studied by this technique. The average cardiac output was 6.0 L. per minute. The average cardiac index was 3.18 L. per minute per square meter, with a standard deviation of 0.73. Simultaneous studies were performed to compare the cardiac output values obtained from an analysis of serial arterial blood samples. Little difference was found between the cardiac output values calculated by the two methods.

Cardiac output determinations by the in vivo method were accomplished in several groups of patients with disease states which have been reported to have associated abnormal cardiac output values. The results of these studies will be presented and the validity of the method will be discussed in detail.

74. THE COMPARISON OF BLOOD PEPSINOGEN IN PATIENTS WITH DUODENAL AND GASTRIC ULCER AND PATIENTS WITH GASTRIC CARCINOMA

I. Arthur Mirsky, M.D., Bernard Fisher, M.D. (By Invitation), and S. P. Harbison, M.D., Pittsburgh, Pa.

Patients with gastric carcinoma frequently exhibit a diminution in gastric secretion, whereas patients with duodenal ulcer usually have an increase in gastric secretion. Since the concentration of pepsinogen in the blood is dependent upon the rate of pepsinogen formation in the gastric mucosa, it became pertinent to determine whether the measurement of the concentration of this proenzyme in the blood could be utilized to distinguish gastric carcinoma from gastric ulcer and/or duodenal ulcer.

Only patients who were scheduled for an upper gastrointestinal operation because of suspected ulcer or carcinoma were studied. A sample of venous blood was drawn from each patient one or two days before the operation and the serum pepsinogen concentration determined by a previously reported procedure. The clinical diagnosis was established in all instances by histologic examination of the tissues removed at operation. One hundred and six patients with duodenal ulcer, 50 with gastric ulcer, and 55 with gastric carcinoma were studied.

Statistical analysis of the data revealed no significant difference between the concentration of serum pepsinogen of patients with duodenal and gastric ulcers. The pepsinogen concentration of patients with gastric carcinoma, however, was significantly ($P < 0.001$) lower than that of patients with gastric or duodenal ulcer. Thus, 50 per cent of patients with carcinoma had values which were lower than the lowest of patients with ulcer, while only 15 per cent of those with carcinoma had values which were beyond the mean of the ulcer group. Although a good deal of overlapping occurred, the data suggest that the level of pepsinogen in the serum may serve as an adjunctive aid in distinguishing gastric carcinoma from gastric and duodenal ulcers.

75. CORRECTION OF METABOLIC DEFECTS IN DIABETES MELLITUS BY CONTINUOUS FRUCTOSE FEEDING

JOHN A. MOORHOUSE, M.D., CHICAGO, ILL.

(INTRODUCED BY ROBERT W. KEETON, M.D.)

This study was undertaken to determine the effects of continuous feeding of fructose over extended periods of time on metabolic disturbances in diabetes. By means of an intragastric tube and standard speed pump, a calorically adequate and nutritionally well-balanced synthetic liquid diet was fed around the clock. This abolished the usual interprandial demand for calories from body stores of sugar, fat, and protein, and also obviated many of the difficulties of the usual type of balance studies. The diet consisted of a solution of carbohydrate (either glucose or fructose in isocaloric amounts), emulsified fat, amino acids, vitamins, and minerals. A constant rate of absorption of metabolites was maintained for days or weeks as desired, and feces were reduced to a minimum. The tolerance of patients for the procedure was excellent.

Eight diabetic patients were studied. Insulin was discontinued, and after a control period, continuous feeding was started. This contained glucose or fructose, but all other constituents were kept constant. Periods of glucose and fructose feeding were compared by suitable blood and metabolic balance studies. Glucose, ketone, lactate, pyruvate, sodium, potassium, and phosphorus, as well as other moieties, were measured frequently. Balances of nitrogen, sodium, potassium, phosphorus, and other moieties were made. In diabetes of moderate severity, fructose feeding was accompanied by a well-defined fall in both blood and urine levels of glucose and acetone. A positive nitrogen balance with retention of phosphate and potassium was observed. (In one patient who gained 9 pounds in weight over a two-week period on continuous fructose feeding, these effects were maintained after return to a normal diet, although before treatment there was loss of weight, marked glycosuria, and ketonemia.) In severe diabetes, fructose appeared to have little or no effect on glycosuria and ketosis. One patient with acromegaly and insulin-resistant diabetes was studied. In this patient ketone levels in the blood and urine fell to normal on fructose feeding, but there was no effect on blood glucose levels. The significance of these findings will be discussed.

76. THE EFFECT OF INTRAVENOUS FAT EMULSION ON TOTAL BILIRUBIN OUTPUT AS A MEASURE OF HEMOLYSIS IN HUMAN SUBJECTS

JOHN F. MUELLER, M.D., MORTON I. GROSSMAN, M.D., AND HUGO C. MOELLER, M.D. (BY INVITATION), DENVER, COLO.

Fever is a common complication of parenteral fat alimentation. Among the many theories as to the cause of the thermogenic response, the occurrence of intravascular hemolysis has been prominently suggested. Anemia has been found in laboratory animals given intravenous fat over long periods of time. Hemoglobinemia is frequently seen in lipemic plasma. It has been suggested that the former is a consequence of the known marked increase in mechanical fragility of red cells in the presence of fat. There are conflicting reports in the literature on studies in humans. Three patients were studied by Creditor, two of whom had slightly elevated fecal urobilinogens after one fat infusion. Shafiroff found no elevation of the urinary urobilinogen after fat.

As previously reported from this laboratory, bile collected via a biliary fistula in intact rabbits showed no increase in bilirubin content after the injection of 10 ml. per kilogram of a 10 per cent fat emulsion. Subsequent studies in rats utilizing a similar technique have revealed elevated values for bilirubin after 40 ml. per kilogram and 20 ml. per kilogram injections of fat as compared with those before. However, with 10 ml. per kilogram doses no increase occurs.

It seemed advisable to settle this problem in human beings. Therefore, analyses of total bilirubin output in bile collected from 8 patients via an indwelling T tube in their common duct following gall-bladder surgery were carried out before and after one 600 ml. fat infusion. A period of 5 to 7 days was allowed to elapse before the study was begun, so the patients were eating well, were afrebrile, and ambulatory. Collections were divided into four-hour periods during the day and a twelve-hour period at night and were continuous for 48 hours before and 48 hours after the infusion. The results indicate that there is no increase in the output of bilirubin in human subjects following the parenteral administration of fat and that in the dosage level used hemolysis could not be demonstrated in vivo. Febrile responses occurred in two of the patients, indicating that mechanisms other than hemolysis were responsible.

77. THE EFFECT OF TILTING ON THE RIGHT ATRIAL PRESSURES OF PATIENTS WITH HEART FAILURE

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Columbus, Ohio

(Introduced by B. K. Wiseman, M.D.)

The effect of upright tilting on right atrial pressure was determined in 24 patients with heart disease of various types. Seven had no failure, the rest had moderate to advanced failure. Ten controls included seven normals and three with massive noncardiac edema.

Supine, a point half the distance from the front to the back of the chest at the fourth right intercostal space was our reference. Upright, the catheter tip in the mid-right atrium (fluoroscopically) was the reference. Pressures were determined in mm. H_2O with a water manometer.

Subjects, with and without heart disease and also with and without edema, having a recumbent pressure of 100 or less had an average pressure drop of 81 when upright. Those with recumbent pressures between 100 and 200 had an average drop of 94, while those with recumbent pressures greater than 200 had an average drop of 166 when tilted. When these high pressures approached normal following treatment, the response to tilt also approached normal. A direct correlation exists between the height of the recumbent pressure and the fall of this pressure after tilting ($r = 0.768$).

It appears that in advanced congestive failure the vascular bed remains distensible. When upright, the increased venous hydrostatic pressure below the diaphragm may exceed venous tone and result in blood trapping, thus reducing right atrial pressure. The effect is probably similar on pulmonary venous pressure. Since elevated pulmonary venous pressure may be a respiratory stimulus, an orthostatic reduction in this pressure would help explain orthopnea and also the relief of paroxysmal nocturnal dyspnea. The characteristics of a venous pressure-volume diagram may partially explain why the highest recumbent pressures have the greatest drops after tilt. The orthostatic decrease in intrathoracic pressure probably accounts for only a small portion of the atrial pressure change.

78. CONCENTRATION OF TRACE METALS IN URINE OF TREATED AND UNTREATED HYPERTENSIVE PATIENTS COMPARED WITH NORMAL SUBJECTS

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With precautions to prevent contamination by extraneous metal, 24-hour urine samples were collected from 15 normotensive subjects whose ages ranged from 6 to 67 years; 6 were Negroes and 8 were men. Similar collections were made from 15 untreated hypertensive patients whose average resting supine blood pressure was 214/134 mm. Hg. Second samples were collected from 8 whose average blood pressure was lowered to 171/104 mm. Hg by 10 to 20 days of ganglionic blockade plus therapy with hydralazine, a metal-binding agent. After all organic matter was digested from 200 ml. aliquots of urine, transition and nearby elements were concentrated with 8-hydroxyquinoline. Subsequently each sample was analyzed in duplicate spectrographically for 11 metals. The precision of the method, as indicated by 10 analyses of one sample, varied from 3.5 per cent for nickel to 12 per cent for chromium.

In order of decreasing weight present, the metals assayed were zinc, manganese, molybdenum, lead, tin, nickel, titanium, vanadium, chromium, cadmium, and silver. The average concentrations in normal urine ranged from 80 ppb (parts per billion) of zinc to 0.8 for silver, with extreme individual values varying as much as tenfold from the mean. No correlation was obvious with age, race, or sex. The most significant changes in the hypertensive group were increases in cadmium and manganese to 40 and 60 ppb, respectively, compared with normal values of 1 and 12. The maximum cadmium concentration was 370, with 4 hypertensive patients having over 37, and 8 over 3.7 ppb. Only 4 normal urines had 3.7 ppb and 8 had less than 1 ppb. The situation was similar but less marked for manganese. Hydralazine lowered cadmium excretion to 6 ppb and manganese to normal.

79. PROBLEMS IN MEASURING EXTRACELLULAR SPACE BY SUCROSE INFUSIONS

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Measurements of extracellular space by constant rate infusion of an "inert" substance (i.e., sucrose, inulin) has been an established method for physiologic studies. It has been assumed that sucrose is equilibrated with the functional extracellular space in the nonedematous patient in 3 to 5 hours. It has also been assumed that sucrose is not metabolized when given parenterally in man.

Our data suggest that sucrose measures a space much larger than that generally attributed to extracellular space; that fluctuations as great as 60 per cent take place in sucrose space in a matter of a few hours; that a constant "space" is not attained even after a 24-hour infusion-equilibration, and that sucrose in some persons is "lost" and probably metabolized.

We measured sucrose spaces on 40 occasions in 28 persons, using a constant rate infusion technique. Most measurements were made at frequent intervals for 6-hour periods or longer and in 8 persons for 24 hours. (1) Sucrose space can change greatly, quickly, and unpredictably. (2) Fluctuations in sucrose space continue over a 24-hour period. Ignorance of factors causing these changes has prevented standardization. (3) Urine recovery of sucrose

in some persons is less than 90 per cent, suggesting that sucrose is metabolized. (4) Studies of bile and saliva in 10 patients have revealed insignificant loss of sucrose through such excretion.

We noted variability in sucrose space of 20 to 60 per cent within 1 to 5 hours even after correction for sucrose loss. Likewise, variability of up to 43 per cent was observed in the same person in similar consecutive studies. Consequently it was not possible to choose a space measurement at any particular interval as being the true "steady state" extracellular space. This variability in successive runs also applied to urine recovery of sucrose in some cases, indicating that loss or metabolism did not occur at a constant rate.

Extracellular space, as it has been measured to date with sucrose, is so variable as to render it of doubtful value as a biologic reference point. The evidence of sucrose being metabolized at a variable rate in some persons indicates that sucrose is not an inert ideal substance for measuring extracellular space.

80. PREFERENTIAL AND ACCESSORY CONDUCTION PATHWAYS IN THE A-V JUNCTION

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(Introduced by Richard Langendorf, M.D.)

Escape beats recorded in clinical electrocardiograms sometimes differ in contour and QRS duration from conducted beats and this is in spite of their having a relatively short cycle length. This suggests a location of the subsidiary pacemaker above the bifurcation of the common A-V bundle. While this, unlike other types of aberrant ventricular conduction, cannot be explained on a functional basis, recent investigations on the structure of the normal A-V junction suggest that "paraspecific" A-V connections may act as preferential pathways to the ventricles in some A-V nodal escape beats. Difficulties and guides in differentiating A-V nodal from ventricular escapes under such circumstances will be pointed out. On the basis of clinical, electrocardiographic, and anatomic facts such a *normal preferential* A-V conduction must be distinguished from an *abnormal accessory* A-V conduction causing the pre-excitation (WPW) syndrome. However, the two may occur in association as will be shown in an actual case. This illustrates the anatomic and physiologic complexity of the A-V junction.

81. SYNTHESIS OF CHOLESTEROL DURING HUMAN PREGNANCY

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The experiments reported here have been undertaken to determine (1) the rate of synthesis of cholesterol in maternal ovaries and in the decidua, (2) the rate of cholesterol synthesis in the fetus, and (3) the placental passage and fetal distribution of maternal dietary cholesterol. Pregnant women who were scheduled for therapeutic abortion for various indications received a single dose of 1-C^{14}-acetate shortly before operation. In some patients cholesterol labeled with tritium was administered orally for periods of 8 to 14 days before surgery. The specific activities of C^{14}-cholesterol and T-cholesterol were determined in the plasma and in various tissues of the mother and the fetus.

The results indicate that the corpus luteum of pregnancy synthesizes cholesterol very rapidly during the seventh to the nineteenth week of embryonic life. Cholesterol in blood and corpus luteum equilibrate relatively fast during the seventeenth and the nineteenth week of embryonic life. In all cases studied, the decidua appeared to be inert in regard to the biosynthesis of cholesterol. The fetal part of the placenta synthesizes cholesterol during early pregnancy, and equilibration between maternal plasma and placental cholesterol occurs during the seventeenth and the nineteenth week of embryonic life. The fetal liver and the fetal adrenals synthesize cholesterol from acetate administered to the mother to an appreciable extent while fetal skin and fetal brain appeared to be inert in this regard. There was no evidence for the local synthesis of cholesterol from acetate in the fetal aorta and its branches. Dietary cholesterol of the mother passes the placental barrier to the fetus to a limited degree during early pregnancy. However, the findings in an anencephalic fetus delivered spontaneously during the thirty-fourth week of embryonic life indicate that maternal dietray cholesterol was rapidly distributed in various fetal tissues in late pregnancy. The adrenals of this anencephalic infant did not synthesize cholesterol from acetate administered to the mother in contrast to the findings in normal fetuses.

82. DEMONTRATION OF STIMULATION OF ERYTHROPOIESIS BY PLASMA FROM ANEMIC RATS USING Fe^{59}

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The presence of a humoral factor in the plasma of anemic rats (bled sufficiently to reduce the hematocrit to less than 20 per cent) that is capable of stimulating erythropoiesis in normal rats has been reported by a number of investigators. Heretofore, assay of this factor has been done by means of serial reticulocyte counts on animals that had received the anemic plasma. In our study, the effect of intravenously administered anemic rat plasma on erythropoiesis in normal rats was determined by measuring the erythrocyte rate of uptake of intravenously injected radioiron (Fe^{59}).

The blood volumes of the rats were taken to be 5 per cent of the body weight. The administration of plasma did not alter this value at the interval when blood samples were withdrawn for the determination of erythropoietic activity. Plasma from normal rats did not stimulate erythropoiesis. A significant response was observed following the injection of as little as 3 c.c. of anemic rat plasma given over a 3-day period. However, it was found that 6 c.c. administered over the same time span elicited the maximum effect upon erythrocyte formation. The optimum time for the injection of Fe^{59} appears to be within a few hours after the final infusion of anemic plasma, and blood samples should be withdrawn during the ensuing 12 hours.

83. REVERSIBLE GLOMERULAR LESIONS IN TOXEMIA OF PREGNANCY AS SHOWN BY SERIAL RENAL BIOPSIES

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Patients suffering from toxemia of pregnancy rarely die. Thus little is known about the pathology of the kidney, especially in pre-eclampsia. A

study was therefore undertaken to correlate the renal pathology with clinical and functional aspects of the syndrome. Fifteen women were studied during pregnancy at the height of the disease process, and 9 of these were restudied again post partum. Rental tissue was obtained by percutaneous needle biopsy. Clinically the disease usually became manifest during the last trimester. All cases had hypertension, and most had edema and proteinuria. In many, severe spasm of the retinal arterioles was striking. No patient had convulsions.

Pathologically the kidney of one patient was normal and two had benign nephrosclerosis. In a fourth, chronic glomerulonephritis with an unsuspected superimposed pyelonephritis was found. This patient later died with multiple microabscesses in the kidney.

Eleven patients had pre-eclampsia. Study of the renal biopsies in this group disclosed swelling of the whole glomerulus. The glomerular basement membrane appeared thickened. This thickening was diffuse, affecting the whole of each and every glomerulus in the sections. There was no cellular proliferation. The thickening appeared to be due to swelling or edema of the basement membrane, rather than to an increased deposition of mucopolysaccharide material, as demonstrated in Hotchkiss-McManus preparations. Proteinaceous material was often present in Bowman's spaces and in the lumina of the convoluted tubules. The tubular epithelium was somewhat flatter than normal, and only mild degenerative changes were seen. There was no abnormality of interstitial tissue and vessels. All but one of the patients recovered after confinement. In those who recovered and who were studied again post partum the histologic appearance of the kidney had reverted completely to normal, and the glomerular basement membrane was thin and delicate.

In one patient, however, with severe pre-eclampsia, definite thickening of the glomerular basement membrane was noted in addition to swelling. Hotchkiss-McManus preparations showed increased deposition of mucopolysaccharide material. After confinement hypertension persisted. The patient developed severe arteriolar sclerosis of the kidney, and the thickening of the basement membrane persisted.

The significance and interpretation of these findings will be discussed.

84. THE DETERMINATION OF CARDIAC OUTPUT BY THE DILUTION METHOD WITHOUT ARTERIAL SAMPLING

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Although the earlier work in this laboratory on the radioisotope determination of cardiac output has been performed by arterial cannulation or puncture, arterial sampling of blood is not required by the general formulation of the method. Studies have been extended to determine cardiac output by focusing a gamma-ray detector externally on large arterial vessels or over the heart itself. This method entails the rapid intravenous injection of a 2 ml. solution of iodinated human serum albumin containing 20 to 60 μc of I^{131} and inscribing a dilution curve externally over the selected site. After a 10- to 15-minute interval a venous sample for the calibration of the curve is withdrawn. The cardiac output, F, can then be calculated by the equation:

$$F = \frac{\text{Injected Dose}}{\text{Area of Curve}}.$$

There are two important factors necessary for the success of the method. The first requirement is that the final dilution calibration measurement originates from the same diluting volume as the primary dilution curve. The second requisite is the correct extrapolation of the descending part of the curve. If the site selected has a number of clearances in different time phases this extrapolation may depart from an exponential decline and will be difficult to predict. This latter problem has been minimized by collimating so that the clearance of either the right or left heart is predominant.

Of 39 correlation studies in patients comparing the in vivo precordial technique with that of the conventional arterial puncture determination, 33 showed an average deviation of ±7 per cent. Inherent technical difficulties of the arterial puncture method, such as the problem of maintaining adequate flow during the long period of time necessary for the correlation study, contributed to the error of 3 of the 6 failures.

Values of cardiac outputs studied ranged from 1.7 to 8.3 L. per minute. All 9 determinations of 3.5 L. per minute or less show excellent correlation. Included in this group were patients in cardiac failure with edema. Since this method eliminates arterial puncture, is performed easily, and gives accurate values, it should prove clinically useful.

85. ERYTHROCYTIN, A NEW CLOTTING FACTOR FROM ERYTHROCYTES; ITS VALUE IN THE STUDY OF HEMOPHILIA

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The erythrocyte contains a potent clotting factor which is liberated upon hemolysis. When added to normal platelet-free plasma, it causes a greater consumption of prothrombin than does platelet extract, thus showing that it not only can completely replace platelets but also is more potent. When added to plasma from a patient with severe hemophilia no detectable consumption of prothrombin occurs, but if increasing amounts of a purified preparation of thromboplastinogen (antihemophilic globulin) are added, the consumption of prothrombin increases proportionately and, on plotting the values, a straight line is obtained.

By using the plasma from a patient with authentic, severe hemophilia as the assay medium, the thromboplastinogen concentration in an unknown plasma can be readily estimated by the increase in prothrombin consumption which its addition induces. The effect of 0.001 ml. of normal plasma mixed with 1 ml. of hemophilic blood can be estimated with accuracy. Since hemophilic plasma contains adequate amounts of PTC and PTA, these factors do no influence the results of the procedure. By means of this new method it is found that the blood of hemophiliacs with a clotting time of 25 minutes or longer contains only a trace of thromboplastinogen, much less than 0.1 per cent of normal. At a concentration of 0.5 per cent, the bleeding tendency is still moderately severe, but above 1 per cent it is mild. The thromboplastinogen concentration in the blood of carriers is the same as in normal individuals. The method is particularly valuable because of its simplicity and accuracy to determine quantitatively the effect of transfusions in hemophilia. On giving fresh plasma, the expected immediate rise in thromboplastinogen is observed. In 24 hours after 500 ml. of plasma is administered, the concentration of thromboplastinogen is still about 0.5 per cent.

86. THE ANTITOXIN RESPONSE OF SCHICK-NEGATIVE RHEUMATIC AND NONRHEUMATIC SUBJECTS TO DIPHTHERIA TOXOID

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Nineteen Schick-negative children with rheumatic fever and eighteen Schick-negative nonrheumatic children were given a "booster" dose of diphtheria toxoid subcutaneously. There were marked individual differences in antibody response measured in vivo in rabbits but no significant differences between the group of rheumatic and nonrheumatic children or between the responses of children with active or inactive rheumatic fever.

Diphtheria antitoxin precipitated in the toxin-antitoxin complex was much less than the amount actually present as measured in vivo.

There appeared to be no significant difference between the rheumatic and nonrheumatic children in the production of nonprecipitating, skin-sensitizing antibodies using the criteria of immediate skin sensitivity to diphtheria toxoid, the relative ability of sera to form specific toxin-antitoxin precipitates in the presence of purified toxin and the relative ability to fix guinea pig complement.

The findings of this experiment furnish further evidence that children with rheumatic fever, either active or inactive, do not respond differently to nonstreptococcal antigens than do nonrheumatic children.

87. THE UPTAKE OF RADIOIRON IN THE BONE MARROW AS A MEASURE OF ERYTHROPOIESIS

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Although numerous studies have been reported on the disappearance of radioiron from the plasma and its appearance in the peripheral erythrocytes, few investigations have been made on the uptake of radioiron by the bone marrow. In the present study, concurrent measurements have been made in the rat on plasma disappearance, bone marrow uptake, spleen uptake, and appearance in the erythrocyte of radioiron administered by intracardiac injection. The maximum dose of radioiron used for the animals which averaged 200 grams in weight was 0.5 μc containing 0.7 μg of iron.

Clearance of radioiron from the plasma was over 95 per cent completed in 6 hours, at which time the bone marrow achieved maximal labeling. The disappearance of the radioactivity from the bone marrow was a linear function of time. The rate of appearance of radioiron in the peripheral erythrocyte was also a linear function of time and the slope was similar but opposite in sign to that for the bone marrow disappearance. These observations furnish strong evidence that the rate of hemoglobin synthesis is constant during the various stages of erythrocytic maturation. The bone marrow disappearance curve indicates a value of approximately 48 hours for the lifespan of the erythrocytic series in this organ. At 48 hours, the bone marrow disappearance and red cell appearance curves change sharply to smaller slopes which are probably due to utilization of storage iron.

The iron taken up by the total marrow erythropoietic mass was approximately 30 per cent of the injected dose. This amount appears in the circulating erythrocytes in 48 hours showing that the storage iron in the marrow is present in a small amount or has a rapid turnover rate.

Approximately 3 per cent of the injected dose of iron appears in the spleen. The rate of uptake and loss from this organ mirrors that of the bone marrow.

88. DEPRESSIONS CAUSED BY HOME EXPOSURES TO GAS AND COMBUSTION PRODUCTS OF GAS, OIL, AND COAL

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Depressions, ranging from mild degrees to melancholia and stupor, have been observed in 50 patients (aged 12 to 78 years and followed 1 to 4 years) to result from daily home exposures to utility gas and combustion products of gas, oil, and coal. Removal of unvented gas ranges and gas refrigerators, leaky electric refrigerators, hot air furnaces, and/or space heaters led to recovery. Removal of gas water heaters, gas driers, all gas piping, and other specifically suspected inhaled, ingested, injected, or contacted materials of petro-, sulfo-, chlorochemical origin was sometimes necessary. Detection requires constant alertness to and recording of patients' complaints and observations. Greater ability to detect escaping gas than other coinhabitants suggests this diagnosis but anosmia frequently makes it unreliable.

Diagnostic observations are made as patients (and families) are removed from and returned to their homes. Improvement follows temporary residence in which patients contact only chemically uncontaminated air, food, drinks, and drugs. As chronic food allergy may cause similar symptoms, this may be investigated concurrently by fasting 4 days followed by single feeding tests with pure common foods. Upon returning home there may be initial burning of eyes, lips, or face, sneezing, coughing, or wheezing associated with nausea in waves, headache, head pain, palpitation, jitteriness, or puffiness, sometimes progressive activity, hyperactivity, and muscular incoordination simulating drunkenness or mania. Increasingly intense waves of depression become continuous, predominating over other symptoms for several days before tapering off with residual aching and fatigue. This follows single exposures in extreme cases. Chronic exposures tend to leave children hyperactive, adults depressed with alternating hyperactivity, mania, or combinations.

Simply turning off the gas line is not diagnostic, for seepage perpetuates symptoms. Households usually contain apparently unaffected members. These points emphasize the significance of small doses and extreme individual susceptibility. Some asymptomatic patients react immediately after entering other homes equipped with gas utilities in good repair or furnaces having leaks between their combustion and hot air chambers, gas-leaking electric refrigerators, or space heaters. Others develop only cumulative effects. Avoidance slowly leads to tolerance.

Intravenous sodium bicarbonate decreases early experimentally or accidentally induced attacks.

89. A MECHANISM FOR DIMINISHED KETOGENESIS IN EXPERIMENTAL LIVER INJURY

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A defect in ketogenesis in human subjects with liver disease has previously been reported by our laboratory. An apparently similar decrease in ketone formation obtains both in the intact animal and the isolated liver slice of rats with experimentally induced liver injury. In an effort to define more precisely the nature of this defect, studies of cell-free mitochondrial suspensions prepared from the livers of ethionine-fed and pair-fed rats were under-

taken. This paper is concerned with the observations made in such preparations. The activities of butyric, pyruvic, alpha-keto-glutaric, succinic, and fumaric oxidases were compared and recorded as QO_2 or ml. O_2 per mg. mitochondrial protein per hour. In addition, the formation of ketones by mitochondria with butyrate and pyruvate as substrates was studied.

Control animals showed a QO_2-butyrate of 74 ± 4 and a QO_2-pyruvate of 68 ± 2 while the ethionine rat suspensions showed a QO_2 of 49 ± 3 and 38 ± 3, respectively. No significant depression of the other oxidase activities was found. Furthermore, control animals formed 0.35 ± 0.03 mM of ketone per mg. mitochondria per hour from butyrate and 0.63 ± 0.05 from pyruvate, while the ethionine group formed only 0.15 ± 0.04 and 0.20 ± 0.03, respectively.

The marked depression of mitochondrial ketogenesis and oxidase activity observed in the fatty acid and pyruvate systems, with no abnormalities of the oxidases intrinsic to the Krebs cycle in animals with liver injury, suggests that the fundamental defect resides in the activition of acetate and its introduction into the Krebs cycle. In view of the known relationships of coenzyme A (CoA) and lipoic acid to these processes, studies relating to these cofactors were made. A striking depletion of hepatic mitochondrial lipoic acid was found in the animals with liver injury. However, in vivo and in vitro addition of lipoic acid and CoA did not reverse the abnormality in oxygen consumption and ketogenesis.

A specific defect in fatty acid and pyruvate oxidation appears to be responsible for defective ketogenesis in animals with experimental liver injury. A similar mechanism may also be responsible for the observations in the patient with liver disease.

90. MYOCARDIAL IONIC EXCHANGE: EFFECTS OF STROPHANTHIDIN IN THE VAGOTOMIZED DOG

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In vitro studies have indicated that acetylcholine applied to cardiac tissue alters its ionic content in a manner similar to digitalis, and it has been postulated that this substance serves an essential role in myocardial contraction.

This study was undertaken to determine whether the cholinergic stimulatory effect of the digitalis analogue, acetyl-strophanthidin, acting upon the cell membrane, is responsible for the myocardial ionic changes and alteration in cardiac function observed in a previous study. Following bilateral cervical vagotomy in 7 anesthetized dogs, the electrolyte changes induced by intravenous infusion of 0.05 mg. per kilogram of strophanthidin were determined, employing the auriculoventricular difference of sodium and potassium as a measure of their myocardial exchange. Simultaneous blood samples were taken from the femoral artery (A) and catheterized coronary sinus (C.S.) at approximately 5-minute intervals for periods up to 60 minutes.

Control A-V differences were not significant (K_A 4.12 meq/L., $K_{C.S.}$ 4.06 meq/L.) (Na_A 157.7 meq/L., $Na_{C.S.}$ 156.3 meq/L.). After infusion, there was an abrupt loss of K from the myocardial cell, reaching in 5 minutes a maximum negative A-V difference of 0.70 meq ± 0.23 meq/L. (K_A 4.54 meq/L., $K_{C.S.}$

5.24 meq/L.) ($P < 0.001$). Myocardial uptake of Na occurred simultaneously, with a maximum positive A-V difference of 7.0 ± 5.3 meq/L. (Na_A 157.4 meq/L., $Na_{C.S.}$ 150.4 meq/L.) ($P < 0.01$). Control values for K and Na were reestablished at 20 minutes. Stroke output declined from 19.8 to 11.1 c.c. Electrocardiographic changes were more severe than in the nonvagotomized dogs. There was no significant respiratory or pH change.

Since these electrolyte and hemodynamic changes do not differ significantly from those of dogs in a previous study having a pronounced vagal effect, it would appear that digitalis directly alters cell membrane permeability to cation and influences myocardial performance without the mediation of acetylcholine elaborated by the vagus.

It has been inferred that the positive inotropism of digitalis depends upon the diminished internal ionic content effected by the movement of potassium from the cell. Since the heart in these normal dogs underwent an increase in internal ionic content due to the greater influx of sodium over egress of potassium, it is postulated that this alteration in the intracellular ionic atmosphere may represent the mechanism for the decline in stroke output in the normal heart under the influence of digitalis.

91. ARTERIAL SOUNDS AS INDICES OF CARDIOVASCULAR FUNCTION

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The sounds heard at the brachial artery have been shown to provide considerable useful information, aside from their common use for the estimation of the blood pressure. Increased blood flow through the arteries compressed by a blood pressure cuff was indicated by enhanced intensity and duration of the sounds. Conversely, reduced flow, whether due to peripheral obstruction or vascular spasm, was associated with muffled sounds. A reduced flow increased the tendency to auscultatory gap; the gap could then be eliminated by increasing the flow through the arm by producing a reactive hyperemia. Patients with atrial fibrillation showed an increased tendency to an auscultatory gap, complicating the estimation of their blood pressure. However, elimination of the gap by reactive hyperemia, combined with the use of an auscultatory-deficit technique (using that cuff pressure value at which half the apical beats were followed by arterial sounds) made it possible consistently to ascertain both the systolic and diastolic pressures in such arrhythmias.

Timing of the arterial sounds by means of the QRS complex made it possible to construct a calibrated pressure pulse contour without arterial puncture. The velocity of the start of the arterial pulse wave was estimated from the lag of onset of the sound after QRS. Epinephrine injections or generalized exertion enhanced the presumptive velocity of the pulse wave and produced a steeper wave front. That these effects were due, at least in part, to increased stroke output was indicated by studies on patients with atrial fibrillation. In these, the celerity and slope of the pulse wave were correlated with the duration of the previous cardiac cycle and therefore with the previous ventricular filling period. These studies indicate the value of clinical investigation of the sounds produced by arteries compressed by a cuff.

92. COMPARATIVE HEMODYNAMIC STUDY INCLUDING CORONARY BLOOD FLOW AND MYOCARDIAL OXYGEN CONSUMPTION IN THE NORMAL, THYROTOXIC, HYPERTENSIVE, AND MITRAL STENOTIC HUMAN SUBJECT

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A series of 46 patients composed of 9 normal persons, 16 with mitral stenosis, 12 with arterial hypertension, and 9 with thyrotoxicosis were studied in the resting postabsorptive state by cardiac catheterization. Cardiac output was measured by the Fick principle and coronary blood flow by the N_2O method. Nine subjects with "mild" mitral stenosis, having a pulmonary arterial mean pressure of 30 or less, were separated from the 7 "severe" whose mean pulmonary artery pressure exceeded 30, to test the assumption that more severe stenosis produced greater hemodynamic change.

Cardiac index was reduced in those with mitral stenosis ($p < 0.01$) and arterial hypertension ($p < 0.01$), but increased in thyrotoxicosis ($p < 0.05$). Left ventricular work was increased in the thyrotoxic ($p < 0.02$) and hyper-

COMPARATIVE HEMODYNAMIC DATA ON VARIOUS PATIENT GROUPS

	NORMAL PERSONS 9	MITRAL STENOSIS 9 (P.A.M. = 30 AND LESS)	MITRAL STENOSIS 7 (P.A.M. = OVER 30)	HYPERTENSIVE PERSONS: 10 CARDIAC OUTPUTS, 12 CORONARY BLOOD FLOWS	THYROTOXIC PERSONS 9
Cardiac index	3.6 ± 0.5	3.1 ± 0.4 −0.5 = −14% $p < 0.05$	2.6 ± 0.6 −1.0 = −28% $p < 0.01$	2.9 ± 0.5 −0.7 = −19% $p < 0.01$	4.9 ± 1.8 +1.3 = +36% $p < 0.05$
Left ventricular work index	4.6 ± 1.0	3.6 ± 0.7 −1.0 = −22% $p < 0.01$	2.8 ± 0.2 −1.8 = −39% $p < 0.001$	5.4 ± 1.1 +0.8 = +17% $p < 0.05$	6.4 ± 1.8 +1.8 = +39% $p < 0.02$
Right ventricular work index	0.9 ± 0.2	1.0 ± 0.3 +0.1 = −11% $p < 0.5$	1.9 ± 1.0 +1.0 = +110% $p < 0.05$	1.1 ± 0.3 +0.2 = +22% $p < 0.3$	1.5 ± 0.6 +0.6 = +67% $p < 0.02$
Coronary blood flow	74 ± 17	71 ± 21 −3 = −4% $p < 0.7$	53 ± 13 −21 = −28% $p < 0.01$	74 ± 15 – –	99 ± 26 +25 = +33% $p < 0.05$
Coronary vascular resistance	1.3 ± 0.4	1.3 ± 0.5 – –	1.6 ± 0.3 +0.3 = +23% $p < 0.2$	1.9 ± 0.4 +0.6 = +46% $p < 0.001$	1.1 ± 0.2 −0.3 = −33% $p < 0.1$
Cardiac metabolic rate for O_2 (c.c./100 Gm.)	8.6 ± 2.2	0.9 ± 2.5 +0.4 = +5% $p < 0.7$	6.5 ± 1.6 −2.1 = −24% $p < 0.05$	9.5 ± 1.8 +0.9 = +10% $p < 0.4$	12.3 ± 3.8 +3.7 = +43% $p < 0.05$
Total peripheral resistance	1,200 ± 165	1,402 ± 320 +202 = +17% $p < 0.2$	1,773 ± 480 +573 = +48% $p < 0.4$	2,240 ± 661 +1,040 = +87% $p < 0.001$	1,345 ± 630 +140 = +12% $p < 0.6$
Total pulmonary resistance	220 ± 53	387 ± 120 +167 = +76% $p < 0.01$	1,126 ± 717 +906 = +412% $p < 0.01$	270 ± 141 +50 = +23% $p < 0.4$	308 ± 167 +88 = +40% $p < 0.2$

tensive subjects ($p < 0.05$) and reduced in mitral stenosis ($p < 0.01$), whereas right ventricular work was increased in mitral stenosis ($p < 0.05$) and thyrotoxicosis ($p < 0.02$). Coronary blood flow per 100 Gm. of left ventricular myocardium was normal in the hypertensive and "mild" mitral stenotic subjects, reduced in the "severe" mitral stenotic subjects ($p < 0.01$), and increased in thyrotoxicosis ($p < 0.05$). Coronary vascular and peripheral vascular resistance was significantly elevated in the hypertensive patients ($p < 0.001$). The myocardial oxygen utilization per 100 Gm. of tissue ($CMRO_2$) was increased in thyrotoxicosis ($p < 0.05$) and decreased in "severe" mitral stenosis ($p < 0.05$), but normal in hypertension and mild mitral stenosis.

When the data for all the patients are grouped together, significant positive correlations are demonstrated between $CMRO_2$ and the left ventricular work index ($p < 0.001$), and $CMRO_2$ and the cardiac index ($p < 0.001$), but not between $CMRO_2$ and heart rate, $CMRO_2$ and right ventricular work index, or $CMRO_2$ and body O_2 consumption per M^2. There was a significant positive correlation between coronary blood flow and left ventricular work index ($p < 0.001$) and between coronary blood flow and heart rate ($p < 0.01$).

93. ELECTROCARDIOGRAMS IN NORMAL SUBJECTS RESEMBLING HIGH ANTERIOR MYOCARDIAL INFARCTION

Sami Said, M.B. (By Invitation), and J. Marion Bryant, M.D., New York, N. Y.

In 1946 Rosenbaum, Wilson, and Johnston described QRS deflections in high precordial leads from patients with symptoms of coronary occlusion as characteristic of myocardial infarction, whereas limb and chest leads from the usual sites were within normal limits. They indicated that similar QRS configurations were not encountered in a small group of normal subjects. Other investigators have reported findings agreeing with this opinion. Subsequently this concept has found wide acceptance in clinical electrocardiographic interpretation and textbooks of electrocardiography.

Electrocardiographic exploration from limb, multiple chest, and esophageal sites has been carried out in a series of normal subjects. In several instances QRS deflections (i.e., prominent relatively broad Q waves) from high precordial sites ($_2V_1$ through $_2V_6$), closely resembling those previously reported as characteristic of myocardial infarction, have been observed. The presence of prominent Q waves in leads from both right and left high precordial sites is a normal variant. Therefore, prominent Q waves from such sites alone, or in conjunction with definite electrocardiographic evidence of myocardial infarction, are not necessarily due to infarction. These findings are of obvious value in the interpretation of the significance of Q waves from indirect epicardial ventricular leads. Thus, they emphasize the difficulty in differentiating between physiologic and pathologic Q waves in unipolar limb and chest leads.

94. EFFECTS OF ALDOSTERONE ON WATER, ELECTROLYTE, AND NITROGEN BALANCES IN ADDISON'S DISEASE

Robert M. Salassa, M.D. (By Invitation), Harold L. Mason, Ph.D., Vernon R. Mattox, Ph.D. (By Invitation), Marschelle H. Power, Ph.D., Alan L. Orvis, Ph.D. (By Invitation), and Randall G. Sprague, M.D., Rochester, Minn.

Increased quantities of aldosterone have been found in the urine of a variety of patients with gross edema, and in the urine and tumor tissue of a patient with an adrenal cortical tumor but without edema. Yet some reports

suggest that the administration of aldosterone will not result in retention of sufficient sodium and water to cause edema. In view of these apparently conflicting reports, it seemed desirable to make further observations of the metabolic effects of this steroid in patients with chronic adrenal insufficiency.

Aldosterone was therefore administered to three patients with Addison's disease in doses varying from 160 to 800 μg per day. Observations were made of effects on electrolyte and nitrogen balances, exchangeable body sodium and potassium, and total body water. Comparative data on the effects of fluorohydrocortisone were obtained. The administration of aldosterone resulted in retention of sodium and chloride, increase in total body water, and increased excretion of potassium. The abnormal electrolyte and water balances of addisonian crisis were corrected. Daily administration of 400 and 800 μg of aldosterone resulted in retention of sufficient sodium and water to lead to the development of mild edema of the ankles and face. No significant changes in nitrogen balance were observed, and there was no gross alteration of cutaneous pigmentation.

The data indicate that whatever physiologic limitation there may be on the electrolyte activity of aldosterone, the steroid in large doses is nevertheless capable of giving rise to mild visible edema.

95. EFFECT OF MILD STEADY STATE EXERCISE ON GENERAL AND CEREBRAL HEMODYNAMICS OF PATIENTS WITH AORTIC INSUFFICIENCY

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(BY INVITATION), CLEVELAND, OHIO

The effect of a mild, steady state of exercise (120 to 130 foot pounds per minute) on the cerebral and general hemodynamics has been studied in 10 patients with isolated aortic insufficiency, clinically not in failure, in the supine position, and employing a variable resistance bicycle ergometer. The amount of work which would ensure a steady state within 10 and maintain it for an additional 20 minutes, as gauged by oxygen consumption, was predetermined the day before the study. Cerebral blood flow (CBF) was determined by the nitrous oxide method of Kety and Schmidt, cardiac output by direct Fick, and pressures transduced via Statham strain gauges. A similar control study on normal subjects (Kleinerman and Sancetta, J. Clin. Invest. **34**: 945, 1955), furnished comparative data.

There were significant increases above the basal, resting state in mean minute ventilation, O_2 consumption per M^2 (131.6 c.c. to 206.4 c.c., $p = > 0.001$), systemic AVO_2 difference (4.91 v.p.c. to 6.56 v.p.c., $p = > 0.001$), and cardiac index (2.71 L. to 3.19 L., $p = < 0.01 > 0.001$). The changes in ventilation and O_2 consumption were not significantly different from those in normal subjects, but there was a significant difference between the two groups in the cardiac index ($p = < 0.01 > 0.001$). Mean total pulmonary vascular resistance increased from 448 c.g.s. to 487 c.g.s. These changes were not significant, but were significantly different ($p = < 0.02 > 0.01$) from the findings in normal subjects, where a significant decrease was noted (242 c.g.s. to 190 c.g.s., $p = < 0.05 > 0.01$). However, pulmonary vascular resistance decreased in all subjects with normal, and increased in those with elevated, resting wedge pressures. Marked increases in pulmonary artery pressure occurred only in the latter subjects.

CBF increased from a mean of 54 c.c. per 100 Gm. per minute to 59 c.c. This was not significant, but was statistically different from the change in the control group ($p = < 0.05 > 0.02$). CMR increased from a mean of 3.0 c.c.

per 100 Gm. per minute to 3.5 c.c. This increase was statistically significant at the 5 per cent level and was different from the changes in the control group. CVR showed no significant change in either group. AVO_2 differences, $AVCO_2$ differences, arterial pH, arterial pCO_2, cerebral R.Q., and arterial O_2 saturations showed no significant changes in exercise as compared with resting values in subjects with aortic insufficiency.

The data may help to explain the infrequent occurrence of syncope in patients with aortic insufficiency.

96. EXPERIMENTAL HISTOPLASMOSIS IN LARGE ANIMALS

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Previous studies from this laboratory have demonstrated that fulminating histoplasmosis could be produced in dogs following intratracheal inoculation of 2 ml. of yeast phase *H. capsulatum*. The present study was conducted to attempt to produce different gradations of disease. Severe disease with 5 deaths was observed in 11 dogs receiving from $\frac{1}{32}$ to 1 ml. of packed cells. A more chronic disease picture was noted in 4 dogs receiving $\frac{1}{64}$ or $\frac{1}{128}$ ml. None of 10 dogs inoculated with 2 ml. of cells was benefited by therapy with ethyl vanillate, mycostatin, Atabrine, or sodium caprylate.

Two horses, cows, sheep, and hogs were inoculated intratracheally with 2 ml. of organisms. Except for fever and cough in some of the 8 animals, clinical signs of disease were negligible. All 8 developed positive skin tests and circulating antibodies. Positive blood cultures were obtained only from the 2 sheep. At necropsy gross lesions were observed in the lungs of 1 sheep and 1 horse.

Fifteen rhesus monkeys were inoculated by various routes. Severe disease followed intratracheal and intravenous inoculation; mild infection after intranasal and no clinical signs of disease after intragastric administration of *H. capsulatum*. Two of the monkeys were pregnant females who died of disseminated disease and positive cultures were obtained from the placentas but not the fetuses. Colored photographs will illustrate the comparative pathologic findings.

97. THE EFFECT OF ANEMIA AND POLYCYTHEMIA ON GLOMERULAR FILTRATION AND RENAL VASCULAR TONE

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Anemia is associated with decreased, and polycythemia with increased, renal blood flow. It is not clear whether these changes result from predominant efferent or afferent glomerular arteriolar vasomotor activity. Forty-two patients without renal disease, whose hematocrits ranged from 17 to 80 per cent and whose blood viscosity varied from 2.5 to 14 times that of water were studied. Renal clearances of para-aminohippurate (P.A.H.) and mannitol were determined to obtain effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), respectively. Effective renal blood flow (ERBF) was estimated in the usual manner after Wintrobe hematocrit was corrected for P.A.H. remaining in the cellular layer. (Hematocrit readings included the buffy coat.) Blood viscosity was determined at 37° C. using an Ostwald pipette. Arterial blood pressure was obtained with a clinical sphygmomanometer. In all polycythemic patients arterial blood oxygen saturation determinations were made. In an attempt to separate influence of hematocrit from that of viscosity, ob-

servations were also made in seven patients in chronic myelogenous leukemia and twice in a patient whose total serum protein was 11.0 Gm. per cent.

An inverse linear relation was found between hematocrit and the GFR/ERBF ($r = -0.95$, 33 patients with hematocrits ranging from 17 to 73 per cent). Similar results were found in secondary polycythemia ($r = -0.88$, in 9 observations with hematocrits varying from 54 to 80 per cent). For a given hematocrit, however, the GFR/ERBF was consistently higher in the secondary group.

In forty-four patients there was an expected linear relation between hematocrit and log of viscosity ($r = 0.98$). Leukemic and the elevated serum protein bloods showed inordinately high viscosity for their hematocrit. In these patients GFR/ERBF correlated better with hematocrit than with viscosity.

These observations suggest that in anemia and polycythemia the predominant factor in controlling renal blood flow may be the vasomotor activity of the efferent glomerular arteriole.

98. EFFECT OF PORPHYRINS ON X-RAY SENSITIVITY OF TUMORS

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(Introduced by Leslie Zieve, M.D.)

Because some porphyrins tend to localize in certain human and animal tumors, various theoretical considerations suggested the possibility that the localized porphyrin might potentiate the effect of x-ray administered to the tumors. Accordingly, x-ray therapy has been given in conjunction with various porphyrins injected (a) into animals bearing transplanted tumors, and (b) into patients with tumors which were considered unsuitable for x-ray because of failure of previous x-ray therapy or because of the known poor responsiveness to x-ray as well as the extensiveness of the tumor.

In 27 patients approximately one-half of the tumors whose size could be measured by direct visualization or palpation showed at least 50 per cent regression during the first 2 to 3 weeks after starting therapy. In 4 of these patients, the tumors previously had failed to respond significantly to x-ray therapy alone. Twelve different types of tumor were represented in this group. The responsive tumors included metastatic fibrosarcoma in the neck, metastatic carcinoid in the abdomen, gelatinous-cell carcinoma of the rectum, rhabdomyosarcoma in the groin, metastatic abdominal adenocarcinoma originating in the ovary, and squamous-cell carcinomas of the tongue, lip, larynx, vulva, and thigh with extensive regional metastases.

Some side effects, limitations, and mechanisms which may be involved in the reactions will be discussed.

99. THE ENHANCEMENT OF THE DEVELOPMENT OF AKR LYMPHOMA BY CELL-FREE FILTRATES OF BRAIN

Steven O. Schwartz, M.D., Harold M. Schoolman, M.D. (By Invitation), and Paul B. Szanto, M.D. (By Invitation), Chicago, Ill.

Cell-free filtrates of leukemic AKR mouse brain were prepared by passage through Seitz filters whose integrity in each case was tested with a broth culture of *E. coli*. Four- to 17-week-old mice of our substrain were inoculated intracerebrally and intraperitoneally. Regardless of the age at the time of

the injection all animals which survived were sacrificed at the age of 22 weeks in order to avoid the possibility of spontaneous leukemia making interpretation of the results difficult.

One hundred and four mice were inoculated intracerebrally and 68 intraperitoneally with filtrates from ten different leukemic mice. Approximately 30 per cent of the animals injected intracerebrally and 33 per cent of the animals injected intraperitoneally developed leukemia in from two to fifteen weeks. These figures include all animals inoculated. If the animals which died during the eight-day postinoculation period as a result of trauma, hemorrhage, or infection are eliminated, the figures for leukemia become 40 per cent and 45 per cent, respectively. None of the littermate controls developed leukemia before the age of 22 weeks. In a control series of over 100 mice injected with filtrates identically prepared from nonleukemic homologous and heterologous mouse brains, leukemia failed to develop in the experimental period.

The enhancement of the development of lymphoma by a cell-free filtrate is interpretable in one of two ways. Either the host response is modified, or an agent capable of accelerating the development of leukemia is introduced. The former is an unlikely supposition considering the quantity of the filtrate used (0.075 c.c. of a less than 0.5 per cent concentration in the intracerebral injections). It is more plausible to assume that development of the disease is enhanced in the already fertile soil of the highly inbred leukemic strain.

100. HEMODYNAMIC EFFECTS OF PENTOLINIUM

RALPH C. SCOTT, M.D., SAMUEL KAPLAN, M.D. (BY INVITATION), WILLIAM J. STILES, M.D. (BY INVITATION), TE-CHUAN CHOU, M.D. (BY INVITATION), RICHARD W. BOOTH, M.D. (BY INVITATION), AND MURRAY B. SHELDON, M.D. (BY INVITATION), CINCINNATI, OHIO

The hemodynamic effects of pentolinium were studied in seven patients. Three patients had systemic hypertension, two had mitral stenosis (one had had mitral valvulotomy), one had chronic cor pulmonale, and one was normotensive.

Cardiac catheterization was performed and the patient was tilted 20 degrees from the horizontal with the feet down. Two resting cardiac output determinations and pulmonary artery, pulmonary "capillary," and brachial artery pressure measurements were obtained. A dose of from 4 to 8 mg. of pentolinium was given intramuscularly. After a time interval of about 30 minutes the cardiac output and pressure measurements were repeated. Due to technical reasons the pressures after pentolinium were not obtained in one patient with hypertension and in the patient with cor pulmonale although their outputs were measured and showed no significant change.

The patients with systemic hypertension exhibited a significant fall in brachial artery pressure, a decline in cardiac output in 2 instances and no change in the third, no change in peripheral resistance in one case and an increase in another, and no change in pulmonary artery and pulmonary capillary pressures.

The patient with mitral stenosis and marked pulmonary hypertension showed a decline in brachial and pulmonary artery pressures, no change in cardiac output, and a decrease in peripheral and pulmonary vascular resistances. The patient who had had mitral valvulotomy presumably did not receive a sufficiently large dose of pentolinium as there was no significant change in pressures.

The normotensive subject exhibited a fall in systemic pressure, a decrease in cardiac output and peripheral resistance, no significant change in pulmonary artery or "capillary" pressures, but a definite increase in pulmonary vascular resistance.

This study indicates that pentolinium does not cause an increase in cardiac output. The observed fall in systemic pressure was due to a decline in cardiac output or peripheral resistance or both. The one instance of decrease of pulmonary hypertension was due to a decline in pulmonary vascular resistance. In three instances there was a decline in systemic pressure but no significant decrease in pulmonary artery pressure, further suggesting that there is independent neural regulation of the pulmonary vascular bed.

101. THE FIBRINOLYTIC ENZYME SYSTEM OF HUMAN PLASMA: STUDIES WITH INTRAVENOUS STREPTOKINASE IN MAN

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In vitro studies suggest that human plasma contains 3 factors which potentially are concerned with fibrinolysis: (1) *plasminogen or profibrinolysin,* the naturally occurring precursor of the proteolytic enzyme of plasma, termed plasmin or fibrinolysin; (2) *plasminogen activator* which probably exists in plasma as an inactive activator-inhibitor complex; when the inhibitor is removed, the released activator kinetically converts plasminogen to plasmin; and (3) *antiplasmin,* which is capable of rapidly inactivating plasmin. Streptokinase (also referred to as SK) the most potent "activator" of the human fibrinolytic enzyme system, appears to produce its effects by: (1) releasing plasminogen activator from its precursor; (2) inhibiting the action of antiplasmin; and (3) adsorbing on fibrin. This latter property allows streptokinase to mediate its effects locally on the fibrin surface, thus enhancing the fibrinolytic as compared with the fibrinogenolytic activity of streptokinase-activated plasmin. The amount of fibrinolytic activity of human plasma, to which SK is added, can be shown at any specific time to be dependent upon (a) the rate of plasminogen activation and (b) the antiplasmin activity. Since both these latter phenomena are related to the SK concentration, the removal of SK by the addition of anti-SK rapidly abolishes the fibrinolytic activity of SK-treated plasma.

Extensive biochemical studies have been made of the effects of streptokinase infused intravenously in man. During the infusion of SK, activation of plasminogen and suppression of antiplasmin activity occurs, and is associated with intense fibrinolytic activity. With the cessation of the SK infusion, the rate of plasminogen activation decreases and the antiplasmin activity rapidly returns to normal. These latter changes are associated with a rapid reduction in fibrinolytic activity. As suspected from in vitro observations, fibrinogenolytic activity proceeds at a much slower rate than fibrinolytic activity. The results obtained are consistent with the in vitro observations described previously.

102. USE OF LARGE DOSES OF VITAMIN K IN LIVER DISEASE

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Vitamin K (5 to 10 mg. daily) has been disappointing in the treatment of a low prothrombin time in patients with liver disease. The effect of large doses of vitamin K in such patients is equivocal although some physicians report that they may actually further increase a prolonged prothrombin time. The benefit of huge doses of aqueous vitamin K and the even better results

of K_1 in patients with hypoprothrombinemia from anticoagulant therapy made us use similar large doses of K in patients with liver disease. Forty-two patients having a prothrombin time of 50 per cent or less of normal were used.

Of the 42 patients receiving 72 mg. of vitamin K (Hykinone), 21 had no change in the prothrombin time after 24 hours; in 17 the prothrombin time expressed as per cent of normal increased from 10 to 50 per cent; while in 4 it decreased from 10 to 20 per cent from the initial level. Of the 42 patients treated with vitamin K_1 (Mephyton) 19 showed no response; 17 showed an increase in prothrombin time expressed as per cent of normal, from 10 to 60 per cent; while 6 showed a decrease of 10 to 30 per cent from the initial level.

Thus, following Hykinone or Mephyton, approximately 40 per cent of the patients responded after 24 hours; in about 20 per cent this persisted during the following day. In about 20 per cent of the patients. improvement was noted 48 hours later. These preliminary observations indicate that large doses (72 mg. of Hykinone or 50 mg. of Mephyton) will improve the prothrombin time in approximately 40 per cent of such patients. In the other 60 per cent not responding to large doses of vitamin K, other factors (Labile factor [V] and the Stable factor [VII]) may be additionally responsible as suggested by studies which are presently in progress.

Conclusion.—These preliminary observations suggest that patients with liver disease whose low prothrombin time does not respond within 24 or 48 hours to large doses of either type of vitamin K, should receive fresh blood or plasma prophylactically against serious bleeding.

103. CHLORPROMAZINE: CHANGES IN HEPATIC HISTOLOGY IN THE ABSENCE OF JAUNDICE

J. L. Sims, M.D., Elvin M. Bremer, M.D. (By Invitation), and Erwin S. Huston, M.D. (By Invitation), Madison and Milwaukee, Wis.

Jaundice, characterized by features suggesting intrahepatic obstruction, occasionally follows chlorpromazine administration. In its absence, liver function tests are usually unaltered during such therapy. Liver biopsies were performed on eighteen patients prior to chlorpromazine treatment and again at its conclusion. Periods of therapy ranged from eight to thirty-three days, doses from 100 to 200 mg. daily. Four patients developed definite infiltration of the portal areas with a mixture of round cells, neutrophils, and eosinophils. Four others showed suggestive but inconclusive change of similar order. None showed bile plugging of the canaliculi. Ten showed no significant change in hepatic histology. No patient became jaundiced, and none developed any abnormality of hepatic function tests. A control series of patients receiving roentgen therapy without chlorpromazine showed no alteration of either hepatic histology or liver function tests.

104. ADULT AND FETAL HUMAN MYOGLOBIN: METHODS OF IDENTIFICATION. REPLACEMENT OF THE FETAL BY THE ADULT COMPOUND IN INFANCY

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By means of the technique of Theorell and deDuve, purified myoglobin, free from admixture of hemoglobin, can be extracted from muscle tissue. The muscle pigment is obtained as acid met-Mb. Normal adult met-Mb (Mb A)

has an absorption spectrum at pH 6.0 which exhibits a "plateau" between the wave lengths 580 and 610 mμ. Electrophoretically, met-Mb A moves slower than met-Hb A in an acid as well as in an alkaline medium. Muscle pigment, prepared from patients with sickle-cell anemia, did not differ spectroscopically or electrophoretically from normal adult Mb. Thus, no "sickle-cell Mb" was demonstrable with these two techniques. Muscle pigment obtained from premature and from newborn infants differs from that of adults. Spectroscopically, acid met-Mb F does not exhibit the "plateau" characteristic for Mb A; this "plateau" is replaced by a distinct minimum at wave length 605 mμ. However, alkaline met-Mb F (pH 8.6) exhibits a "plateau" between wave lengths 490 and 530 mμ which is lacking in the spectral pattern of alkaline met-Mb A. Electrophoretically, Mb A and Mb F move differently, the fetal compound moving faster than the adult pigment in acid as well as in alkaline medium.

At birth, only Mb F is demonstrable. Replacement of Mb F by Mb A takes place gradually (27 specimens examined). In infants aged 2 to 5 months, both Mb A and Mb F may be present. After the age of 6 months, only Mb A was encountered. Thus, the period of disappearance of the fetal red-cell pigment from the circulation coincides approximately with the period of disappearance of the fetal type of pigment in muscle tissue.

105. DEGLUTITION PRESSURES IN ESOPHAGEAL DISORDERS

Hubbard W. Smith, M.D. (By Invitation), E. Clinton Texter, Jr., M.D. (By Invitation), and Clifford J. Barborka, M.D., Chicago, Ill.

Deglutition pressures were recorded from three points 5 cm. apart in the supradiaphragmatic esophagus or gastric cardia in 15 normal subjects, 12 patients with hiatus hernia, and 6 patients with cardiospasm. An adaptation of the open-tipped catheter technique of Quigley was used. The most distal level studied was located immediately above the diaphragm. This was in the gastric segment in patients with hiatus hernia. Duration and amplitude of the deglutition waves were measured following a satisfactory barium swallow, and the values in the three groups were compared.

Both the duration and amplitude of the deglutition pressure waves in cardiospasm were found to differ significantly from the normal ($p < 0.01$). This was also true at the distal level in patients with hiatus hernia, in whom pressures were significantly below normal in the herniated segment. No significant difference in pressures at the distal level was found between patients with hiatus hernia and patients with cardiospasm.

Pressure changes within the lumen of the gut, especially the esophagus, are well correlated with propulsive activity. These data indicate a defective propulsive motor mechanism in cardiospasm and in the supradiaphragmatic gastric segment in hiatus hernia. The relationship of this disorder to the genesis of symptoms and complications will be discussed.

106. A HEPARIN-PRECIPITABLE FRACTION OF HUMAN PLASMA

Richard T. Smith, M.D., Minneapolis, Minn.

(Introduced by Robert A. Good, M.D.)

A qualitative change in circulating fibrinogen of rabbits, manifested by cold precipitation in the presence of heparin, occurs as a regular response to intravenous injection of gram-negative bacterial endotoxin. The studies to

be reported are concerned with the characterization and measurement of a similar fraction in human plasma. This fraction was demonstrated in human heparinized plasma as a flocculant, opaque precipitate appearing 20 to 30 minutes after chilling to 4° C. The precipitate redissolved readily upon warming to 37° C. No precipitate was observed in heparinized serum or citrated plasma. The precipitation reaction appears to be dependent upon the presence of calcium ions, to vary inversely with ionic strength of the plasma.

A semiquantitative method of estimating this fraction, based upon its insolubility at 4° C. under standard conditions, has been employed in serial studies of patients with various diseases, and in measurements of a group of normal persons. The mean value for 368 normal adults was 0.134 ± 0.002 Gm. per cent, the range from 0.04 to 0.26 Gm. per cent. Marked increase in amounts of this fraction with levels as high as 1.4 Gm. per cent, were encountered in the plasma of patients with acute rheumatic fever, rheumatoid arthritis, bacterial endocarditis, and bacterial meningitis. Serial measurements showed that the initially high levels return rapidly to normal upon spontaneous inactivation of these diseases or during effective antibiotic or steroid therapy.

Evidence that this fraction is closely related to fibrinogen is based upon its absence from serum, spontaneous clottability on prolonged standing at 4° C., or on addition of thrombin, and migration parallel to fibrinogen on paper electrophoresis. In normal persons the heparin-precipitable fraction amounted to 30 to 70 per cent of the clottable fibrinogen value; however, in ill patients the fraction often equaled or exceeded fibrinogen. The precise relationship of this fraction to fibrinogen is still under study. The data available, however, suggest that the heparin-precipitable fraction represents a labile form of circulating fibrinogen which increases in amount as a reflection of the presence of inflammatory disease, the magnitude of change being roughly in proportion to the severity of the process.

107. EFFECT OF ENVIRONMENTAL TEMPERATURE UPON CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN THE RABBIT

Wm. A. Sodeman, M.D., and John T. Logue, M.D. (By Invitation), Columbia, Mo.

Atherosclerosis was produced in rabbits by a standard feeding method (rabbit chow impregnated with cholesterol). Appropriate controls were fed the same diet without added cholesterol. The animals were divided into two groups, one maintained at an environmental temperature of 45° to 50° F., the other at 80° to 85° F. for two weeks preceding and throughout the feeding periods. Blood samples were obtained for cholesterol levels on the first, twenty-first, and forty-second days. On the same, sera protein patterns were studied by paper electrophoresis. Staining was done for protein and lipids. At the termination of the study the animals were sacrificed, the aortas dissected out, and freshly mounted on flat paraffin sheets, intimal surfaces upward. Photographs were taken to show the extent and location of plaque formation.

Results showed remarkable reduction in plaque formation in the animals kept at the higher temperature levels in spite of higher total cholesterol and ester values in this group. There were also differences in lipoprotein patterns with a higher sharper peak in the beta range in the 80° to 85° animals.

The findings indicate that heat stress in the rabbits, under the conditions studied, produced a profound effect upon the extent and distribution of aortic atherosclerotic plaque formation. Possible mechanisms in the metabolic factors involved will be discussed.

108. THE EFFECT OF DICUMAROL ON THROMBOPLASTIN FORMATION

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The delay in conversion of prothrombin to thrombin as a result of Dicumarol therapy is well recognized. It has been adequately demonstrated that this delay is due largely to a depression of so-called stable factor by the Dicumarol. It is less well recognized that Dicumarol has a profound effect on thromboplastin formation, and an attempt has been made to clarify the means by which Dicumarol suppresses intrinsic thromboplastin formation. Utilizing the thromboplastin generation test, it could be confirmed that the serum of patients receiving Dicumarol therapy was responsible for the impaired thromboplastin generation. Substitution of the plasma or platelets from patients receiving Dicumarol therapy gave normal results. This could be explained by any one of four possible mechanisms: (1) stable factor is necessary for the conversion of the precursors of thromboplastin to active thromboplastin, (2) Dicumarol depresses plasma thromboplastin component, (3) an inhibitor is present in the serum fraction, or (4) some previously unrecognized factor is depressed by Dicumarol therapy.

With one notable exception, the results support the concept previously suggested that stable factor is necessary for thromboplastin formation. Contrary to expected results, on storage of the Dicumarol serum there was initially an increase in its activity in the system; but within a few days it became much less active. There did not seem to be any difference whether the serum was stored at room temperature, 6° C., or in the frozen state. The initial increased activity on storage might be expected if stable factor were responsible; however, if such were the case, it would be anticipated that this stored serum would retain the increased activity over a much longer period. There was no evidence for an inhibitor or depression of plasma thromboplastin component.

109. INTERIM REPORT ON THE EFFECTS OF LONG-TERM ESTROGEN THERAPY IN MEN UNDER FIFTY YEARS OF AGE WITH A PREVIOUS SINGLE MYOCARDIAL INFARCTION

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For three years a long-term study has been in progress on the possible efficacy of estrogens in the treatment of human coronary atherosclerosis. During this time, 160 patients with clinical coronary disease have been under observation. Of these, 72 meet the criteria of the definitive study, i.e., are males under 50 years of age with only a single proved myocardial infarction, which occurred less than one year prior to initial observation, and which is unassociated with gross hypertension, renal disease, diabetes mellitus, and/or other heart disease. These patients were divided into two groups on a "double-

blind'' basis: estrogen-treated and placebo. Analysis of these two groups as of Aug. 31, 1955, revealed them to be practically identical with respect to mean age at infarction, mean height and initial weight, mean duration after infarct when study was begun, mean pretreatment plasma cholesterol-phospholipid-lipoprotein levels. During the last 15 months, estrogen dosage has been 10 mg. per day orally of mixed conjugated equine estrogens (Premarin). This induced gynecomastia, decreased libido and potency, varying in degree, side effects which only rarely resulted in discontinuation of therapy. No significant toxic effects were noted from long-term medication with estrogens. As of Aug. 31, 1955, mean duration of observation was 19 and 22 months (placebo and treated groups, respectively); 25 and 26 patients, respectively, have been on medication for one year or longer; 13 and 8 patients, respectively, for two years or longer. Within 1 to 2 months, estrogens at the 10 mg. dosage level induced significant increases in serum phospholipid and α-lipoprotein levels, with unchanged or decreased cholesterol concentrations, resulting in decreased $\frac{\text{cholesterol}}{\text{phospholipid}}$ ratios, alterations which tended to persist during subsequent months of treatment. As of Aug. 31, 1955, 4 deaths had occurred in the placebo group, and one in the estrogen-treated group, results which do not yet permit definitive conclusions as to therapeutic efficacy, but warrant continuation and expansion of the study.

110. THE NEPHROTOXIC PROPERTIES OF RABBIT ANTISERUM PREPARED BY IMMUNIZING RABBITS WITH PURIFIED HUMAN GLOMERULAR BASEMENT MEMBRANE OR DOG GLOMERULAR BASEMENT MEMBRANE

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Nephrotoxic sera was prepared by immunizing rabbits with dog glomerular basement membrane after the method of Krakower and Greenspon.

In a similar manner purified human glomerular basement membrane was prepared from isolated human glomeruli and used to immunize rabbits. The antihuman membrane serum was injected into dogs intercardially in the amount of 1.5 c.c. per pound.

The dogs were kept in metabolism cages and daily samples of urine were obtained pre- and postinjection and analyzed for protein and a microscopic examination made for cells and casts. Only dogs whose urine was essentially protein-free were injected. Blood for chemical determination of nonprotein nitrogen, albumin, globulin, and cholesterol were obtained prior to injection. A unilateral nephrectomy was performed on all the dogs, usually one or two days after injection, and the kidney obtained for histologic studies. About seven to nine days after injection the nephrectomized dogs were sacrificed and again the bladder urine collected for analysis, blood for chemistry and serology, and some of the organs, including the kidney, for histologic study.

In vitro studies on the complement-fixing properties of purified dog and human glomerular basement membrane against their antisera have been carried out.

It is concluded that (1) normal rabbit serum injected into dogs does not cause nephritis; (2) antidog basement membrane sera injected into dogs produced nephritis; (3) antihuman basement membrane sera injected into dogs produces a nephritis which is similar, if not identical, to the antidog basement membrane-induced nephritis using clinical, chemical, and pathologic criteria.

It is conceivable that nephrotoxic properties of serum prepared from human basement membrane (of the purity obtained) results from a cross-reac-

tion in vivo due to the presence of an immunologically similar, or identical, common antigen in dog and human basement membrane. This last concept is supported by evidence of cross-reaction in vitro using a complement-fixation test.

111. OBSERVATIONS ON THE EARLY STAGES OF SENSITIZATION TO FOREIGN PROTEINS

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Although it is commonly stated that injections of soluble heterologous protein antigens in normal animals are followed by the development of "immediate" or Arthus-type hypersensitivity, there have been several reports suggesting that during the early stages of such sensitization there may occur reactions characteristic of delayed, or tuberculin-type, hypersensitivity. It has been found that following a single intradermal injection of crystalline ovalbumin in normal rabbits, a delayed form of cutaneous hypersensitivity appears abruptly within a few days, prior to the appearance of precipitating antibody in the circulation. The corneal and dermal reactions to test doses of the antigen have been studied and appear to differ from both the classical tuberculin and Arthus reactions, but in general resemble the former more closely. The magnitude and route of administration of the sensitizing dose of antigen have been found to be critical.

112. SECONDARY ALDOSTERONISM

I. THE METABOLIC AND ADRENOCORTICAL RESPONSES OF NORMAL MEN TO HIGH ENVIRONMENTAL TEMPERATURES

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Previous reports (Conn and associates, 1946 to 1949) have indicated that acclimatization to heat involves increased adrenocortical secretion of a "desoxycorticosterone-like" steroid without concomitant increases of other adrenocortical steroids. The present experiments indicate that: (1) exposure to heat results in secretion of large quantities of aldosterone; (2) the increased aldosterone secretion is stimulated independently of other adrenocorticoids and, therefore, is not controlled by endogenous ACTH; (3) the metabolic adjustments which occur are due to increased quantities of aldosterone; (4) the mechanism can be "turned off" by administration of sodium but not of potassium.

Balance studies were performed on four normal adult men exposed to the following conditions: (1) control diet of average composition in temperate climate (7 to 18 days); (2) reduction of dietary sodium to 35 meq. per day in temperate climate (7 days); (3) same diet as (2) but exposure to heat (14 to 23 days); (4) same as (1) (7 days).

Adrenocortical Response.—1. Aldosterone increased slightly under condition (2) but greatly under condition (3), frequently to 65 μg per day (normal below 3 μg). 2. 17-hydroxycorticoids and 17-ketosteroids remained unchanged under condition (2). Both rose slightly during the first two days of

condition (3). 17-hydroxycorticoids then returned to base line and 17-ketosteroids dropped significantly below base line. 3. Infusion of saline solution decreased aldosterone abruptly but did not affect the other urinary steroids.

Metabolic Response.—1. During exposure to heat, sodium and chloride virtually disappeared from urine. Na/K ratio in sweat fell sharply. This ratio in saliva was not consistently depressed. 2. Two subjects established sodium equilibrium within two days by promptly reducing sweat sodium concentration (a function of aldosterone). Total adjustment could not be attained by the other subjects because they were unable to reduce sufficiently the loss of sodium in sweat. This appeared to be end-organ (sweat gland) failure, since aldosterone production was comparable in all subjects.

The kidney could not reduce potassium excretion sufficiently to offset the losses in sweat, with consequent negative K balance.

It is believed that these data will be helpful in clarifying the mechanism of control of aldosterone secretion.

113. ADDISON'S DISEASE DUE TO HISTOPLASMOSIS OF THE ADRENAL GLANDS

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Histoplasmosis involves the adrenal glands relatively frequently but its role in the development of Addison's disease, in the absence of terminal manifestations of disseminated histoplasmosis, has not been clarified. Only one case of histoplasmosis with symptoms and signs of Addison's disease relieved by hormone therapy has been reported. The following case of arrested disseminated histoplasmosis with adrenocortical insufficiency was confirmed at necropsy and further establishes the causative relationship of histoplasmosis to Addison's disease.

The patient was a 50-year-old white man with ulcers at the base of the tongue and on the lips and a bone-marrow reaction from which *Histoplasma capsulatum* was cultured. Hepatomegaly with granulomatous infiltrations at biopsy was also present. An acute crisis of adrenocortical insufficiency occurred and was relieved by hormone therapy. Ethyl vanillate therapy was then administred for histoplasmosis with subsequent disappearance of mouth lesions and hepatomegaly and all other signs of active infection. Kepler-Power water test and eosinophil response following ACTH and Adrenalin administrations were characteristic of adrenocortical insufficiency. Regulation was carried out with DOCA and lipoadrenal extract for two years, and with cortisone for two years. Death occurred outside the hospital. Enlarged and necrotic adrenal glands containing many yeast cells characteristic of *Histoplasma capsulatum* were found at necropsy. The lesions were consistent with arrested histoplasmosis.

This case establishes the occurrence of Addison's disease due to histoplasmosis of the adrenals. The relative frequency of histoplasmosis, coccidioidomycosis, and tuberculosis as causes of Addison's disease with granulomatous infection of the adrenals, has not been determined. The possibility is discussed that either: (1) Addison's disease due to *Histoplasma capsulatum* may be found wherever histoplasmosis is common with a relative frequency corresponding to the exposure of the population to the disease; or, (2) that Addison's disease occurs infrequently as a result of histoplasmosis of the adrenals because most such patients die early in the course of the disseminated infection. A possible corollary of the latter is that ethyl vanillate therapy may have prolonged life in the two cases so far reported sufficiently to allow for the development of Addison's disease.

114. A COMPARISON OF THE HEMOLYTIC AND BLOCKING ACTIVITIES OF ANTISHEEP RED CELL SERUMS

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Studies with I^{131} trace labeled rabbit antibodies to sheep red cells have resulted in the development of a technique for measuring the ability of antiserums to block the uptake by red cells of the labeled antibody. Various antisheep red cell serums have been analyzed for blocking activity using a 50 per cent blocking unit and labeled antibodies specific for two different antigenic fractions of the red cell, the heat labile (heterophil) and the heat stabile (isophil). These results have been compared with the hemolytic activity as measured spectrophotometrically in 50 per cent hemolytic units. In general the antiheterophil antibody has contained higher ratios of hemolytic to blocking activity than the anti-isophil antibody. This increased hemolytic activity of the antiheterophil antibody was found to reside in a fraction with a molecular weight higher than the bulk of the blocking antibody. This conclusion is based on the results obtained from ten antiheterophil serums which were subjected to ultracentrifugal fractionation at an average field of 105,000 × g. for 200 minutes. In every case the ratio of hemolytic to blocking activity increased progressively from the top through the middle to the bottom fraction. Evidence obtained from absorption curves, although not conclusive, indicate that the hemolytic and blocking antiheterophil antibodies may have identical specificity although they have different molecular size. If identity of specificity can be established, it would indicate that in this one system, at least, the increased biologic activity of an antibody (in this case, hemolysin) is dependent upon an altered physical property of the antibody (in this case, molecular size or shape). Such a concept would have important implications to the mechanism of allergic reactions.

Continued thrice weekly injections of rabbits with sheep red cells resulted in the development of serums with progressively lower ratios of hemolysin titer to heterophil blocking titer. This finding has theoretical implications to the clinically accepted practice of treating patients suffering from hay fever with repeated injections of pollen allergen.

TABLE I. RATIOS OF HEMOLYSIN TO BLOCKING UNITS IN ULTRACENTRIFUGAL FRACTIONS OF ANTIHETEROPHIL SERUMS AND IN ORIGINAL WHOLE SERUM

SERUM FRACTION	7	8	9	10	11	12
Whole	405	158	117	684	131	66
Top	107	50	23.5	–*	42	17
Middle	338	103	100	1130	106	53
Bottom	947	291	264	1714	321	168

*Blocking titer below level of accurate measurement.

TABLE II. RATIOS OF HEMOLYSIN TO HETEROPHIL BLOCKING UNITS AFTER THRICE-WEEKLY INJECTIONS OF 10 PER CENT SHEEP RED CELLS INTO RABBITS

WEEKS OF IMMUNIZATION	ANIMAL NO. 1	2	3	4	5	6	MEAN AND S.D.
1	941	440	679	470	619	519	611 ± 185
2	472	400	396	536	381	284	411 ± 97
3	271	462	196	223	176	209	256 ± 106
4	74	189	35	160	64	100	103 ± 169
6	82	–	26	132	27	85	70 ± 93

115. DETERMINATION OF CARBON^{14}-LABELED ACETATE UTILIZATION BY TUBERCLE BACILLI

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The C^{14} acetate utilization of both virulent and avirulent tubercle bacilli was studied by adding C^{14} methyl-labeled acetate to the microorganisms in Conway dishes and determining the C^{14} uptake and conversion to $C^{14}O_2$ by the bacilli. The results obtained were correlated with other signs of metabolic activity including growth as measured in nephelometric units, triphenyl tetrazolium chloride (TTC) reduction, and staining characteristics.

The acetate utilization was found to be greatest during the logarithmic phase of growth, diminishing through the arithmetic phase. The amount of utilization was inversely proportional to the density of the culture. In contrast with these findings, TTC reduction was diminished neither by measuring bacterial density, nor in the "exhaustion" phase after cessation of growth. Since TTC can be reduced anaerobically as well as aerobically, it was concluded that acetate utilization is dependent upon oxygen available to the bacterial cell. During the lag-phase before growth commenced, the acetate utilization was increased with increasing density of the culture, but there was no TTC reduction. This seems to lend weight to the theory that some essential metabolite must be synthesized before growth can occur.

To demonstrate that C^{14} acetate uptake and conversion really measured some metabolic activity of the bacilli, these were submitted to varying temperatures before and during the utilization period. After exposure to 4° C. the bacilli took up and converted only very small amounts of acetate, if allowed to remain at that low temperature. Under these conditions they did not reduce TTC. If they were exposed to 4° C. for 3 days and then incubated at 37° C. with C^{14} acetate, they exhibited significant acetate uptake and conversion to CO_2, even though they still did not reduce TTC. Bacilli which were exposed to temperatures higher than 55° C. for 30 minutes prior to the utilization period showed very little or no acetate utilization or TTC reduction.

Streptomycin added to a culture inhibits growth and acetate utilization immediately. When isoniazid (INH) is added to tubercle bacilli, growth continues for approximately one more generation and then ceases, and the bacilli eventually become nonacid-fast. In the first 24 hours after addition of INH, more $C^{14}O_2$ is produced and less C^{14} incorporated into the cell than in untreated bacilli. This seems to indicate decreased synthesis of cell substance with isoniazid.

116. MUSCLE COMPOSITION IN HUMAN MUSCULAR DYSTROPHY

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(INTRODUCED BY WILLIAM B. SEYMOUR, M.D.)

The cause of muscular dystrophy is unknown. One concept holds the disease is an intrinsic defect in muscle energy metabolism, through genetic error. Denny-Brown, alternately, feels that the defect lies in faulty myofibril regeneration. We wish to report biochemical studies performed on biopsied rectus muscle in ten dystrophics compared with normal controls. Patients

were selected with mild or moderate disease hoping that any specific defect would be better appreciated in early rather than in scarred tissue of advanced dystrophy. Noncollagenous nitrogen was used as a parenchymal muscle reference base to correct for nonfunctional replacement. The fibrous replacement of dystrophic muscle is reflected in the striking increase in collagen nitrogen of approximately 400 per cent. This increase was roughly proportional to the severity of the disease. Fatty infiltration, a characteristic of all muscle degeneration, is particularly noticeable in early muscular dystrophy, as shown by a fat increase of about 300 per cent.

The principal protein in muscle is myosin. If myofibril degeneration takes precedence, the myosin might be decreased proportionately more than muscle enzymes. A uniform decrease averaging 25 per cent was found. The previous study on enzymes in dystrophy showed a decrease in some of the glycogenolytic enzymes (Dreyfus and associates, J. Clin. Invest. **33**: 794, 1954). Therefore, in our study over-all glycolysis was compared with activity of enzymes not involved in the Meyerhof-Emden cycle. Creatine kinase, which phosphorylates creatine, was studied since deficient formation of creatine phosphate might be consistent not only with creatinuria but with decreased energy stores. Creatine kinase activity was definitely decreased but to no greater extent than myosin content or the energy degrading enzyme ATP-ase, both being reduced to about 75 per cent of normal. Succinic dehydrogenase activity showed essentially no reduction. The most marked reduction was seen in over-all glycolysis where lactic acid production was only one-half normal. This relatively greater decrease in glycolysis may be more apparent than real but suggests further study to determine the rate limiting step or steps in these linked glycolytic reactions. These methods also allow quantitative characterization, in a small biopsy, of the chief parenchymal protein myosin, and delineation of the degree of replacement fibrosis and lipomatosis.

117. THE COMPARATIVE SENSITIVITIES OF GRAM-NEGATIVE BACILLI TO THE ANTIBIOTICS

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Therapy of infections due to gram-negative bacilli, other than the Salmonella, remains a serious problem. The vast majority of these infections are caused by five groups of organisms: *Escherichia coli, Aerobactor aerogenes,* Proteus, *Pseudomonas aeruginosa,* and *Pseudomonas species.* The data to be presented resulted from a study of the comparative sensitivities to antibiotics of over 200 pathogenic strains of these bacilli. All tests were made by the tube dilution method and each strain was isolated from an acute infection. The data will be introduced by three short case reports in which correlations between the results of the tube dilution test and clinical results were clearly demonstrated. The order of activity of each of the antibiotics against each of the five species of bacteria then will be presented. Optimal combinations of drugs for the treatment of infections due to Proteus and *Pseudomonas aeruginosa* will be shown. The data also will be presented in such a way as to demonstrate how these results may be of aid in selecting chemotherapy for fulminating infections on a statistical basis in cases in which the clinician cannot wait for the results of laboratory studies. Finally, the instances in which there have been significant changes in the resistance of certain of the gram-negative bacilli to the various antibiotics, and the mechanisms by which these changes came about will be discussed.

118. INVESTIGATIONS ON THE ROLE OF THE CENTRAL NERVOUS SYSTEM IN SHOCK PRODUCED BY ENDOTOXIN FROM GRAM-NEGATIVE MICROORGANISMS

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A clinical problem of increasing magnitude, induced partly by antibiotic therapy, is the occurrence of shock in patients caused by the liberation of endotoxin from gram-negative bacteria. The abnormal physiologic activity produced by endotoxins from various species of gram-negative microorganisms is strikingly similar. Purified endotoxin from *Brucella melitensis* or *Escherichia coli* injected intravenously into dogs caused the animals to become hyperpneic, walk about dragging their hindlegs, and manifest a precipitous drop in systemic blood pressure. The portal venous pressure increased up to four times within the first five minutes. Within five minutes, massive evacuation of the bowels and vomiting occurred, followed by profuse bloody vomitus, and tenesmus with bloody diarrhea. Coma ensued in three hours, and death within four to eighteen hours. On autopsy, prominent features were congestion of the liver, submucosal bleeding of the duodenum and jejunum, and edema of the gall bladder. A fall in blood pressure, a rise in portal pressure, and comparable pathologic changes were obtained in dogs anesthetized with Nembutal.

Shock was not prevented in dogs chordotomized at the sixth cervical vertebral level, with and without vagotomy. A prompt fall in blood pressure from endotoxin also occurred in the decapitated dog preparation.

Anesthetized cats displayed a similar although more variable response to endotoxin than dogs. When the entire spinal cord in cats was destroyed by pithing, endotoxin still caused shock. Using the cross-circulation method of Penner and Klein in dogs, which has been presumed to separate the cephalic and the body circulations, shock and the classical pathologic changes were still observed following the injection of endotoxin into the body.

These experiments do not eliminate the role of the central nervous system in the progression of shock caused by endotoxin. However, the investigations do not support the thesis that shock results primarily from the effect of endotoxin on the brain. The locus of endotoxin activity is under further study. Preliminary data on animals indicate that endotoxin has a diffuse effect on vasculature, and a significant direct action on the liver, which modifies the pattern of shock.

119. PRELIMINARY STUDIES OF THE EFFECT OF SELENIUM CYSTINE ON LEUKEMIA

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Previous studies indicate that a decreased availability of l-cystine may have important effects on leukocytes. Leukemic leukocytes exhibit a more rapid turnover of l-cystine than normal leukocytes and may therefore be more susceptible to depletion of this amino acid than normal leukocytes. Selenium cystine, which is identical with cystine except that selenium replaces sulfur within the molecule, is effective in low concentrations in decreasing the influx of l-cystine into leukemic leukocytes in vitro. The effects of this compound on five patients with leukemia were therefore studied.

A rapid decrease in the total leukocyte count and spleen size occurred in two patients with chronic myeloid leukemia and in two patients with acute leukemia. Selenium cystine had no effect on one patient with chronic monocytic leukemia. The most striking effects were observed in acute leukemia. In one patient the leukocyte count decreased 200,000 cells per cubic millimeter within one week and as much as 75,000 cells per cubic millimeter within 24 hours. In chronic myeloid leukemia the immature granulocytes disappeared more rapidly than mature granulocytes. Responses were obtained in patients who were refractory to other therapeutic agents. Thus one patient with acute leukemia who had become refractory to cortisone, aminopterin, and 6-mercaptopurine responded to selenium cystine. This patient subsequently appeared to reacquire sensitivity to 6-mercaptopurine.

Toxic manifestations attributable to selenium cystine included nausea, vomiting, anorexia, alopecia, and loss of fingernails, all of which were reversible when the drug was discontinued. No hepatic or renal damage was encountered.

The mechanism of action of selenium cystine on leukocytes is not known. Preliminary experiments indicate that cystine accelerates certain enzymatic reactions of leukocytes and that selenium cystine interferes with this accelerating effect. These results are further evidence of the importance of cystine in the metabolism of leukocytes.

120. TEN-YEAR PROGNOSIS OF ACUTE MYOCARDIAL INFARCTION

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A ten-year follow-up study was made of 211 individuals who lived more than two months after their first myocardial infarction. The majority of those who died did so within the first five years. More young people live more than ten years after an infarction than do those in the older age groups. The patient who has made a good functional recovery lives longer than those who are disabled by congestive heart failure, angina pectoris, or a cerebrovascular accident. Three-fourths of the patients made a complete or partial economic recovery. The major cause of death, 88 per cent, was cardiovascular disease.

121. OBSERVATIONS ON WATER AND ELECTROLYTE BALANCE IN THE COURSE OF ACCELERATED HYPERTENSION TREATED WITH ANSOLYSEN

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There is experimental and clinical evidence suggesting that water and electrolyte metabolism may be altered in the course of arterial hypertension. For this reason, observations have been made on the balances of water, Na, K, Cl, and N in 3 patients with accelerated hypertension receiving Ansolysen therapy.

Studies were made during a control period, and during a period of blood pressure reduction by Ansolysen. In 2 patients muscle obtained by biopsy was analyzed for water and mineral content.

The first patient was a 36-year-old woman with Grade IV retinopathy, relatively normal renal function, and no evidence of cardiac failure. Water, Na, Cl, and N balances were unchanged during the control and drug periods. However, there was a significant negative K balance throughout both periods. The second patient was a 51-year-old man with Grade IV retinopathy, moderately impaired renal function, but no cardiac failure. There was a slight loss of sodium during the control period, with a gain during therapy. Changes in water, K, and N balance were insignificant. Muscle biopsies showed some decrease in extracellular space and K content at lower range of normal. The third patient was a 42-year-old woman with Grade III retinopathy, marked impaired renal function, and absence of cardiac failure. During the control period, there was a loss of Na and K, with no alteration in H_2O balance. During the drug period no significant changes occurred. A muscle biopsy showed a marked decrease in cellular K content.

These data are suggestive of a K-losing state during the course of malignant hypertension. The presence of renal impairment renders difficult the interpretation of water and sodium balance. No specific water and electrolyte shifts were associated with the depressor effects of Ansolysen.

122. THE ROLE OF PROGESTERONE IN THE BIOGENESIS OF STEROID HORMONES IN MAN

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Fourteen microcuries of 4-C^{14}-progesterone were given intramuscularly to a female patient with Cushing's syndrome due to a metastatic carcinoma of adrenal origin. Urine was collected for the following four days and subjected to hydrolysis and extraction procedures to recover steroid hormone metabolites. Twenty-six per cent of the radioactivity administered was recovered in the pooled ketonic, nonketonic, and phenolic fractions. During the 4-day period of urine collection the patient excreted approximately 130 mg. of 17-ketosteroids and 130 mg. of corticoids. Expired air was collected during the first 9 hours and no radioactivity was detected in the carbon dioxide.

Crystalline pregnanediol was isolated from the nonketonic fraction with a specific activity (SA) of 142.3 μc per mM which was 15 per cent of the SA of progesterone administered. Radiochemically pure crystals of tetrahydrohydrocortisone (THF), tetrahydrocortisone (THE), etiocholanolone, and cortisol were obtained from the ketonic fraction, and each steroid contained the carbon-14 label. The SA values found were 0.149, 0.149, 0.131, and 0.128 μc per mM, respectively. These values represent approximately 0.014 per cent of the SA of the administered C^{14}-progesterone, and about 0.1 per cent of the SA of the pregnanediol isolated. In addition to these normal steroid metabolites, several unidentified steroids containing radioactivity were detected in the ketonic fraction. Since crystalline samples were not obtained, the identity and purity of these steroids are not known.

The results provide further evidence that human adrenal tissue can carry out hydroxylation at C_{11}, C_{17}, and C_{21} of the tetracyclic ring. It is evident that the adrenal can utilize progesterone, as well as cholesterol, as a precursor for cortisol, THF, THE, and etiocholanolone. The relative importance of progesterone as a precursor of adrenal cortical hormones cannot be determined from this study.

123. SOME CLINICAL AND PATHOLOGIC OBSERVATIONS ON THE INTRACRANIAL COMPLICATIONS OF STAPHYLOCOCCAL SEPTICEMIA

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It has long been recognized that the brain and meningeal complications of systemic staphylococcal infections are common and lethal. Among 55 cases of staphylococcal septicemia seen at the Cincinnati General Hospital since 1940, 21 (38 per cent) were demonstrated by lumbar puncture or autopsy to have meningitis or cerebritis. The incidence among cases with endocarditis was 54 per cent, or five times as great as when the heart valves were not infected (10 per cent).

Patients with this complication usually exhibited stupor or coma, stiff neck, and variable other neurological findings such as muscle-twitching and hemiplegia. Convulsive seizures, either generalized or focal, were uncommon.

In the spinal fluid pleocytosis was variable, from a few to 1,000 white blood cells per cubic millimeter. Red blood cell leakage into the subarachnoid space was also common, but counts above 10,000 cells per cubic millimeter were not encountered. Cerebrospinal fluid protein was often moderately elevated, but the level of sugar was not depressed even in the four cases in which smear or culture of the fluid was positive for *Staphylococcus aureus.*

Pathologically the commonest findings were leptomeningitis, usually mild, and miliary septic infarction of the brain with the formation of miliary abscesses adjacent to occluded vessels. Abscesses larger than 1 cm. in diameter were not encountered.

Although cases of staphylococcal septicemia with cerebral involvement exhibited an over-all mortality of 81 per cent, it was often surprisingly easy to abolish the manifestations of meningitis and cerebritis with vigorous systemic antibiotic chemotherapy using penicillin alone or combinations of various drugs. A total of eight patients were successfully managed in this manner. Four of the eight, however, died later of other effects of the septicemia. Penicillin and bacitracin were occasionally employed intrathecally, but eradication of intracerebral infection was achieved in other instances without injection of drug into the subarachnoid space.

Meningitis and cerebritis occurring in the course of staphylococcal septicemia with or without endocarditis should not, therefore, be considered a uniformly dire complication of this disorder, for it can occasionally be eradicated by energetic drug therapy.

124. THE EFFECT OF CALCIUM ON THE FIBRINOLYTIC ACTIVITY OF HUMAN PLASMA

SLOAN J. WILSON, M.D., HELEN E. HEATH, A.B. (BY INVITATION), AND
WILLIAM E. LARSEN, M.D. (BY INVITATION), KANSAS CITY, KAN.

The fibrinolytic activity of plasma was observed without an anticoagulant (native plasma) and when the calcium-combining anticoagulants were used, namely, sodium citrate, sodium oxalate, and the Versene EDTA. Fibrinolysis was determined by collecting whole blood in siliconed glassware with and without anticoagulants, cooled immediately in ice water, centrifuged at 5° C., and separated into 1.0 ml. aliquots of plasma. Thrombin was added for thorough conversion of fibrinogen to fibrin. The fibrin was determined at

0, 24, and 48 hours after incubation at 37° C. The fibrin clot was determined quantitatively in mg. per cent. The entire procedure was carried out under sterile conditions to eliminate bacterial fibrinolytic activity.

In 49 normal human adult subjects the fibrin decrease in 48 hours in native plasma was minimal as follows: 21 subjects, no decrease; 20 subjects, 0 to 30 mg.; 7 subjects, 31 to 60 mg.; 1 subject, 75 mg.

In 28 subjects the fibrinolytic action was observed in native plasma and with added oxalate. In the native plasma the fibrin decrease in 48 hours was less than 60 mg. in all but one subject. The lytic action in the oxalated plasma was more marked in 12 of the 28 subjects; 6 ranging from 60 to 200 mg. decrease, 3 ranging from 201 to 300 mg. decrease, and complete lysis in 3 subjects. EDTA had a greater effect in increasing the lytic action. Sodium citrate increased the lytic action but to a lesser degree than sodium oxalate or EDTA. Wherever calcium was added to the plasma in excess to the amount combined, the fibrinolytic activity was that observed in native plasma.

It is concluded that the fibrinolytic activity of plasma is increased by sodium citrate, sodium oxalate, and Versene EDTA. The increased fibrinolytic activity can be converted to normal by adding calcium to plasma containing these anticoagulants. Calcium is important in the inhibition of plasmin (fibrinolytic) activity.

125. COLLAGENASE, AN ENZYME OF THE PANCREAS

SIDNEY E. ZIFFREN, M.D., AND ROBERT T. HOSIE, M.D. (BY INVITATION),
IOWA CITY, IOWA

It is commonly accepted that the pancreas secretes the following proteolytic enzymes: trypsinogen and chymotrypsinogen, both activated by intestinal juice to trypsin and chymotrypsin, and carboxypeptidase. Chymotrypsin and carboxypeptidase do not digest collagen, while commercially available trypsin digests only minute quantities, since the point of action of these enzymes on the protein molecule is dependent upon preliminary hydrolysis of the collagen. The purpose of this paper is to demonstrate that another enzyme is present in the pancreas, collagenase, which digests collagen. Studies were carried out on the pancreatic fluid of dogs with surgically created pancreatic fistulas and on the fluid of humans with fistulas and pancreatic cysts. The method of measuring the activity of this enzyme was by quantitatively assaying hydroxyproline after autoclaving the undigested portion in an acid medium as described by Neuman and Logan.

The pancreatic juice of dogs has a high activity on collagen as does the juice of human fistulas and fluid from human pancreatic cysts. The ability of commercially available trypsin to digest collagen is slight. This ability is destroyed by heating to 95° C. for 4½ minutes while its tryptic activity is virtually unaltered. Collagenase activity has been demonstrated to be destroyed by such heating and would tend to indicate that another enzyme is present in the commercial preparations.

The action of this enzyme is similar to the collagenase secreted by the *Cl. histolyticum* and by maggots. It is probable that the violence of pancreatitis is related to the release of this enzyme which has a profound action on collagen. This is also the probable cause of the dermal digestion so often seen in the skin of patients with a pancreatic fistula. Our concept of pancreatic disorders may undergo a change in line with the action of this newly described enzyme.

126. EVALUATION OF MITRAL AND AORTIC VALVULAR DISEASE BY LEFT AURICULAR PUNCTURE

HERBERT B. ZIMMERMAN, M.D., AND BERNARD BERCU, M.D., ST. LOUIS, MO.

(INTRODUCED BY ROBERT J. GLASER, M.D.)

After careful selection of patients with mitral stenosis for valvuloplasty, there still remains a certain number who at operation are found to have a significant amount of mitral insufficiency. In the authors' experience, this figure is about 6 per cent. In addition, an unknown number of patients with a tight mitral stenosis may be incorrectly diagnosed as having a significant degree of mitral insufficiency and be denied the benefits of surgery.

The most logical approach to this problem seemed to be a study of the circulatory dynamics at the mitral valve area by means of left auricular puncture. This procedure was carried out by a method previously described by Björk. A polyethylene catheter was passed through the needle into the left auricle and then into the left ventricle to obtain the pressures in these chambers. Criteria for evaluation of the anatomic deformity of the mitral valve were established by correlating the findings obtained by left auricular puncture with the valvular changes described by the surgeon at operation. The diastolic pressure gradient between the left auricle and ventricle was used as an index of the degree of mitral stenosis, while the amount by which the height of the pressure of the v wave in the auricular pressure curve exceeded that of the c wave indicated the degree of mitral insufficiency. Fifty-five patients with mitral stenosis were studied by this method. Only one patient failed to show the type of valvular defect predicted. The procedure was easily performed with little discomfort to the patient. In two instances the left auricle was not entered, presumably due to the severe dorsal kyphosis present in these two patients. A right hemothorax occurred in one patient, but was readily evacuated by thoracenteses.

The clinical evaluation of the anatomic valvular deformities of patients with combined mitral and aortic valvular disease is also extremely difficult since both aortic stenosis and mitral insufficiency produce left ventricular enlargement. Left auricular puncture in seven such patients has provided the necessary information for a more exact understanding of each separate valve lesion.

127. A STUDY OF SERUM GAMMA GLOBULIN IN DYSTROPHIA MYOTONICA

HORACE H. ZINNEMAN, M.D., AND JEROME ROTSTEIN, M.D., MINNEAPOLIS, MINN.

(INTRODUCED BY WILLIAM W. STEAD, M.D.)

During endocrinologic and metabolic studies in 12 patients with dystrophia myotonica, it was noted that the electrophoretic patterns of the sera of 10 patients showed abnormally elevated beta globulin, whereas the sera of 7 patients showed abnormally low levels of gamma globulin. The abnormality of gamma globulin is the object of this study.

Antigenic challenges to 3 of these patients with the polysaccharides of *D. pneumoniae,* types I and II, and typhoid-paratyphoid vaccine resulted in an increase of the gamma globulin fractions to normal levels and formation of demonstrable antibodies.

Since these findings did not suggest an abnormality in the production of gamma globulin, the possibility of an accelerated rate of destruction of this

globulin was examined. The turnover rates of intravenously injected I^{131}-labeled human gamma globulin were determined in six patients with dystrophia myotonica and six normal adults. The turnover rates of I^{131}-labeled human serum albumin were determined in the same subjects. We found that the half life of I^{131}-labeled gamma globulin was shortened in 6 patients with dystrophia myotonica (5.4 days to 8.6 days, with a mean of 7.6 days), as compared with 6 normal adults (8.6 to 13.6 days with a mean of 10.5 days). The half life of I^{131}-labeled human serum albumin in these patients (mean value of 10.2 days) was not essentially different from 6 normal adults (mean value of 9.6 days).

These results suggest that the tendency in patients with dystrophia myotonica to diminished serum gamma globulin is due to an increased catabolism of this globulin. Since the albumin turnover rates in these patients are within the normal range, the abnormality of the metabolism of serum gamma globulin does not appear to be part of a generally increased protein catabolism.

Erratum

In abstract 55 entitled "The 'Corrected' Bromsulfalein Test; A Comparison With the Cephalin-Cholesterol Flocculation Test and the Thymol Turbidity Reaction in Jaundiced Patients," by F. W. Hoffbauer, Charles Metzler, and Ellis Benson, which was published in the November issue of the Journal, page 827, the "greater than" and "less than" signs were omitted from the following sentences which should read as given below. "The results in 111 icteric patients (1′ serum bilirubin $>$ 1 mg./100 ml.) have been analyzed." (Paragraph 1.) "In parenchymal jaundice, tests were termed 'informative' (BSP, corrected, $>$ 20 per cent; CC, 3+ or 4+; TT, $>$ 7 units) or 'misleading' (BSP, corrected, $<$ 10 per cent; CC, 0 or 1+; TT $<$ 5 units). In obstructive jaundice results were termed: 'informative' (BSP, corrected, $<$ 10 per cent; CC, 0 or 1+; TT $<$ 5 units) or 'misleading' (BSP, corrected, $>$ 20 per cent; CC 3+ or 4+; TT $>$ 7 units)." (Paragraph 2.)

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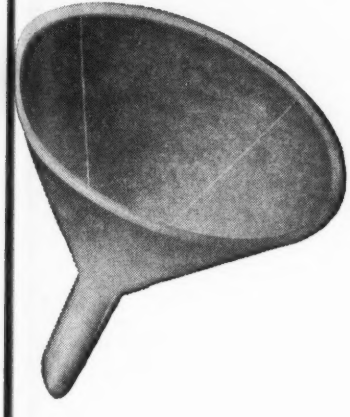

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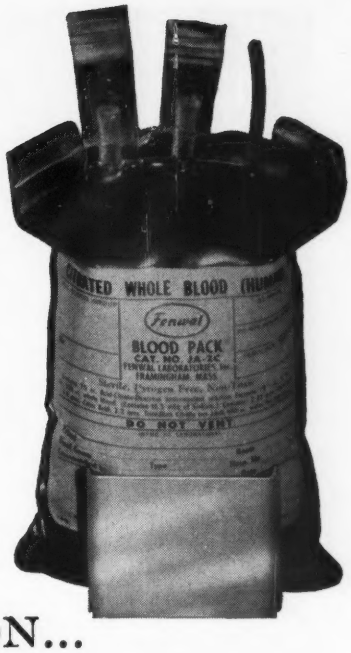

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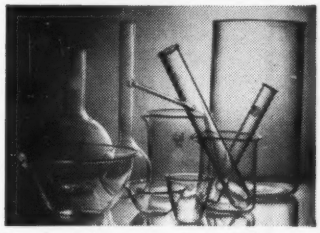

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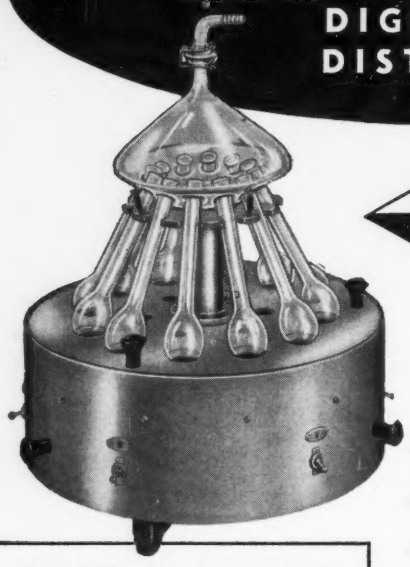

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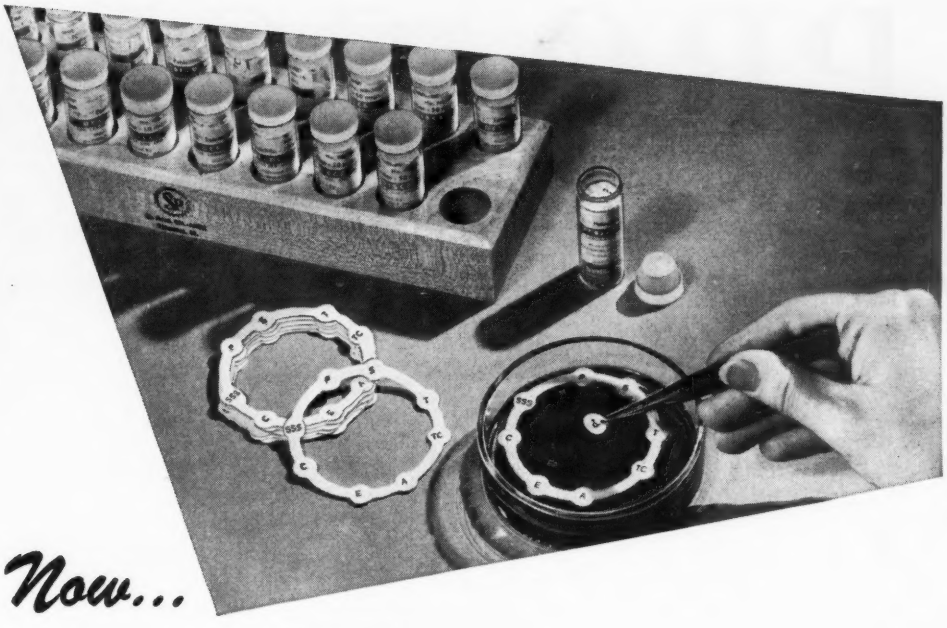

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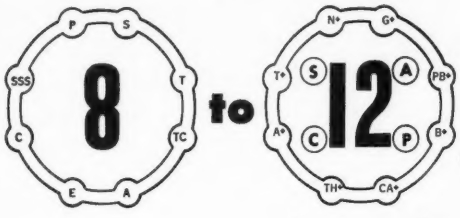

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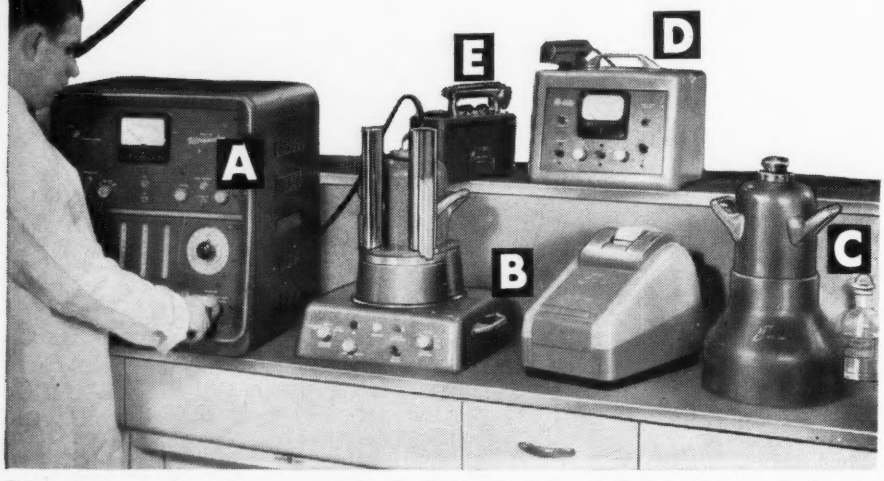

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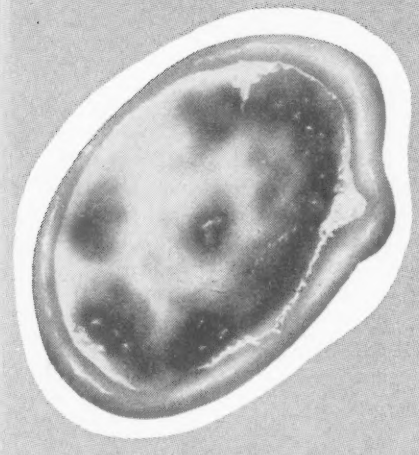

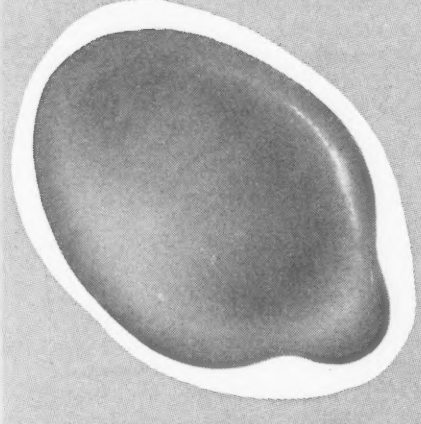

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VOL. 1—X + 378 p., 113 fig., VI col. plate, 1954. $13.50

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In 1955 we shall publish

VOL. 2

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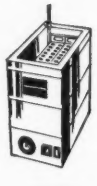

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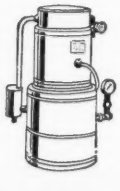

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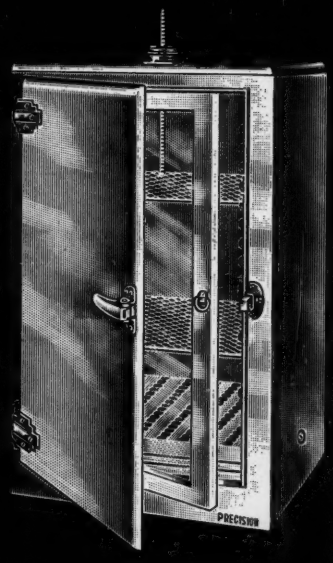

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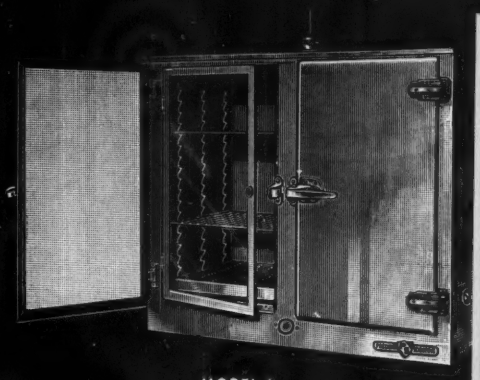

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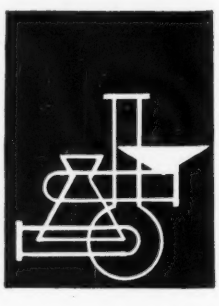

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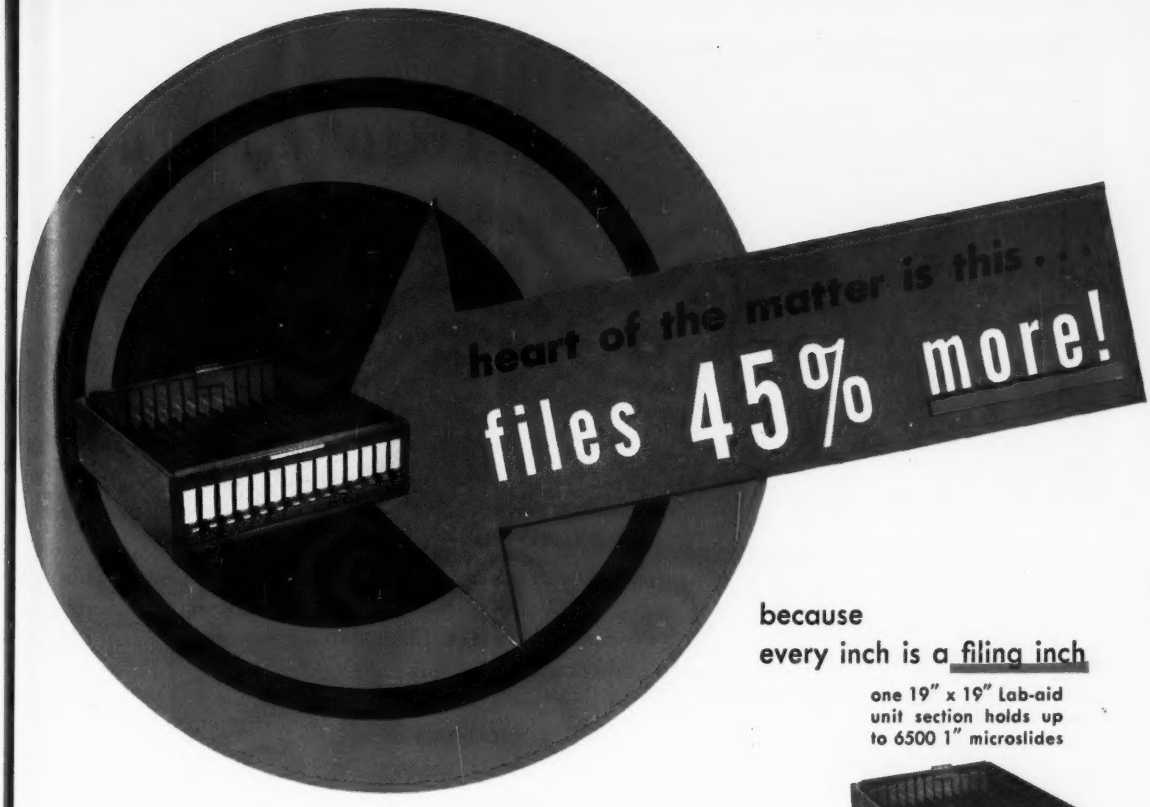

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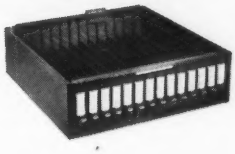

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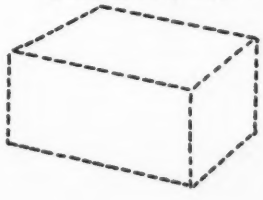

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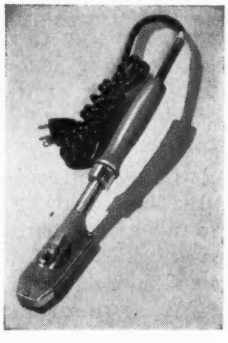

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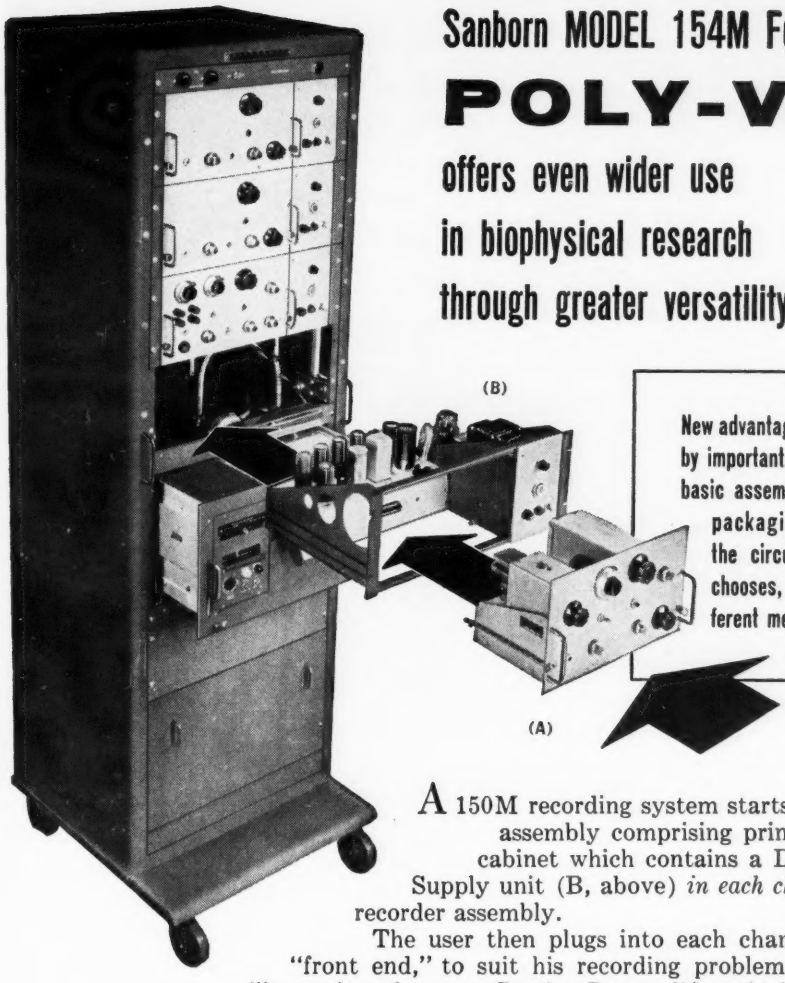

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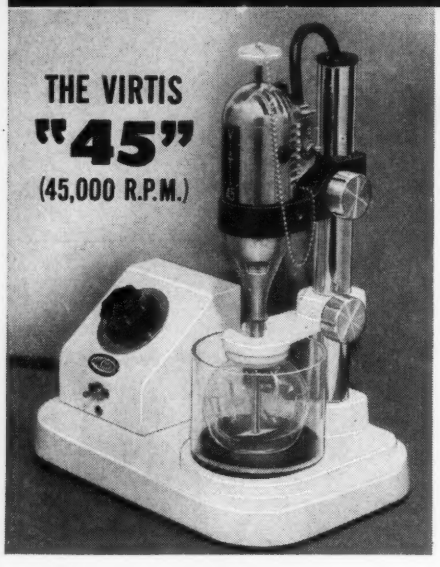

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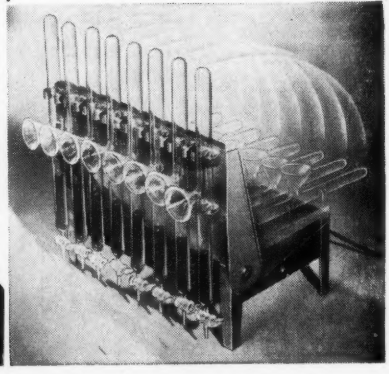

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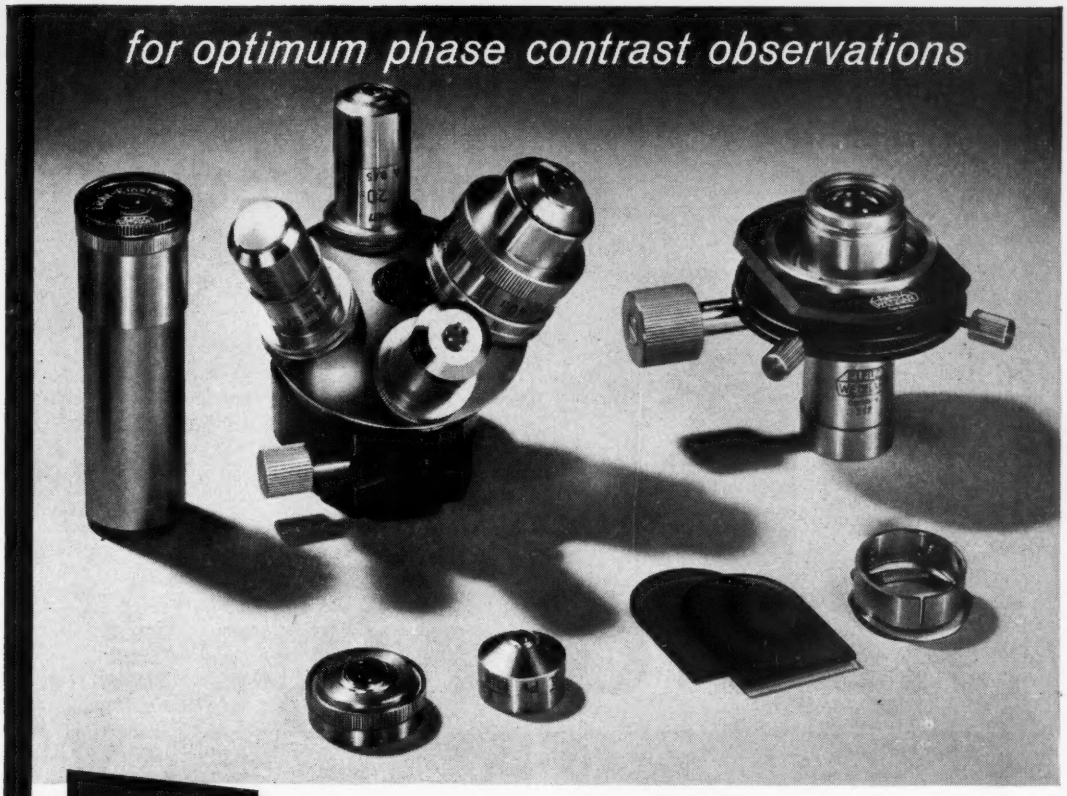

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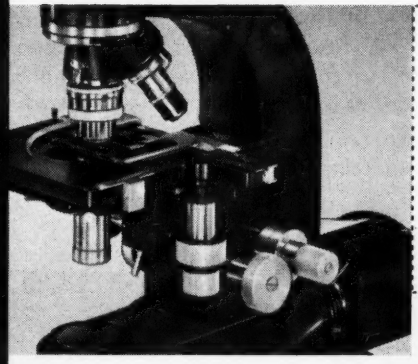

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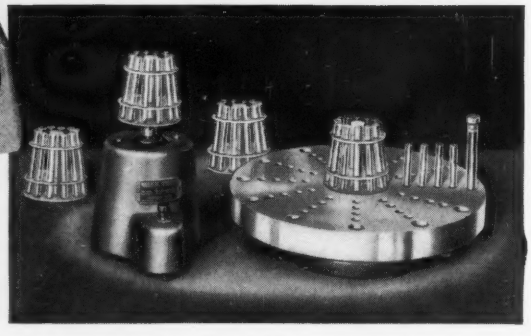

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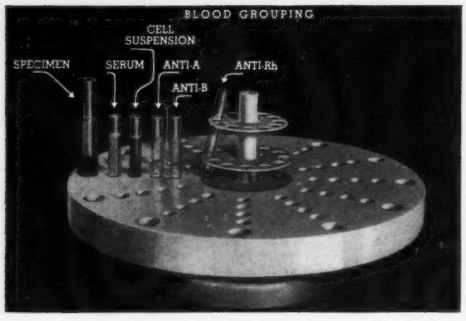

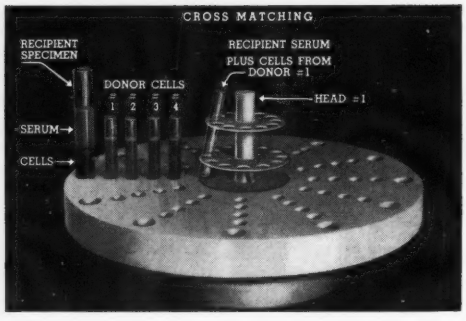

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**New England Journal of Medicine 250: 71–73 (1/14/54), Alan Richardson Jones, M.B., B.S. (Lond.)*

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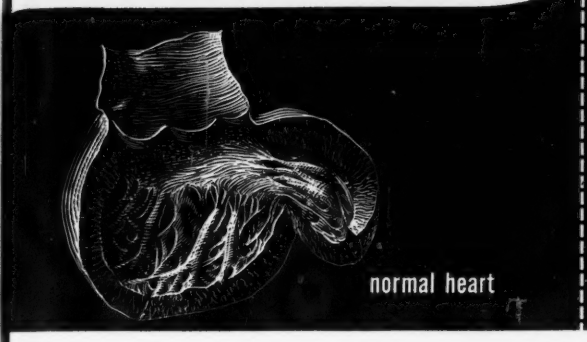

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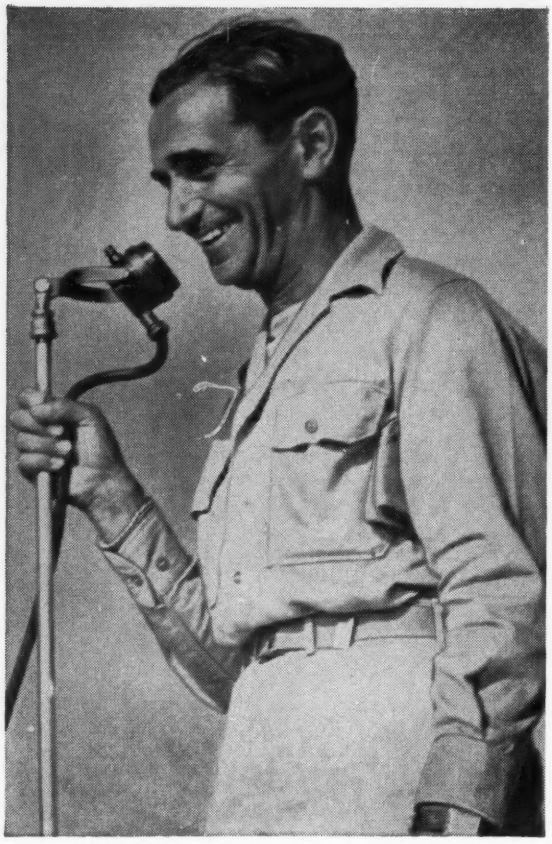

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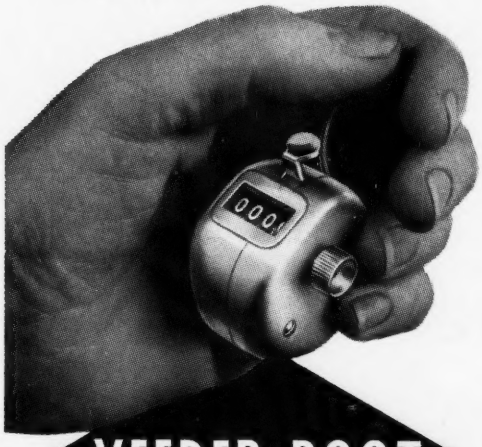

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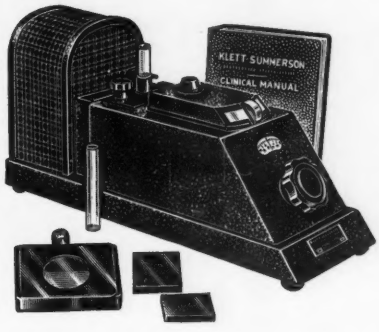

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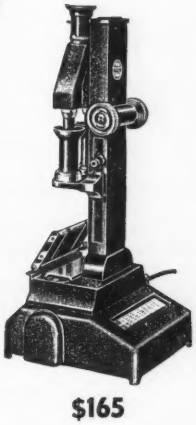

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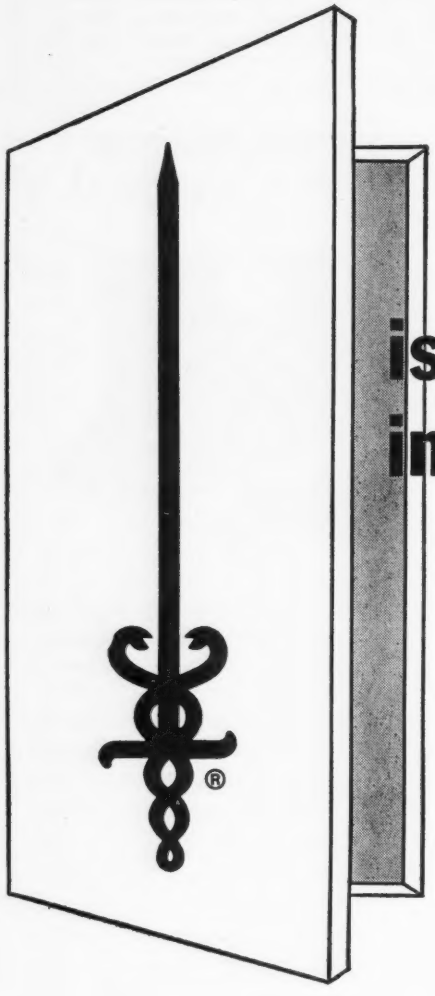

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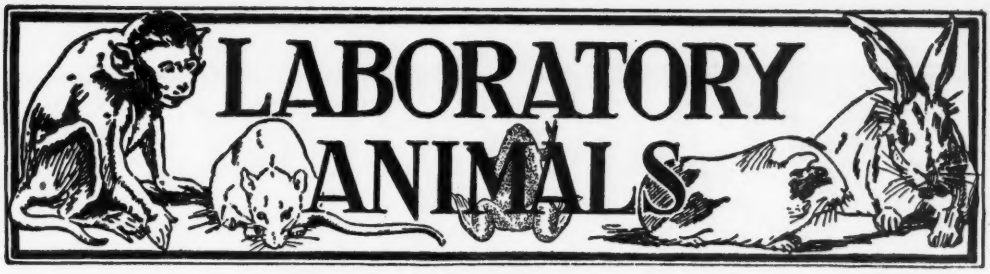

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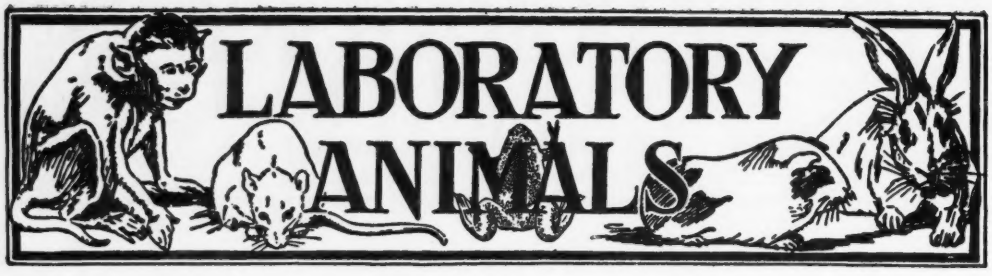

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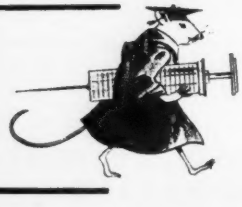

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1. Antibiotics & Chemotherapy, Vol. IV, No. 9, Sept, 1954 2. Antibiotics & Chemotherapy, Vol. III, 409, 1953
3. Antibiotics & Chemotherapy, Vol. II, 30, 1952 4. Nebraska M. J., 37: 251, 1952.

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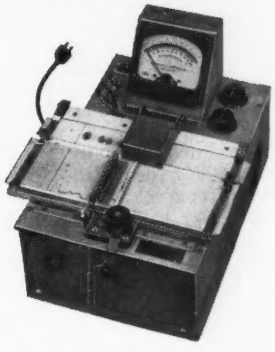

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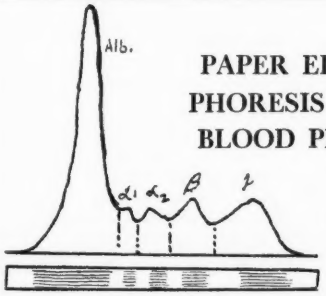

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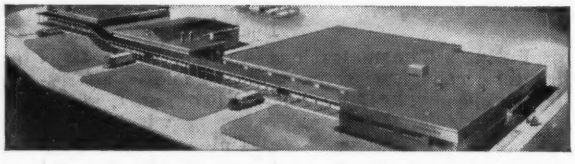

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